Peptide-Binding G Protein-Coupled Receptors: New Opportunities for Drug Design

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Abstract: Over the last decades distinct members of the G Protein-Coupled Receptor (GPCR) family emerged as prominent drug targets within pharmaceutical research, since approximately 60 % of marketed prescription drugs act by selectively addressing representatives of that class of transmembrane signal transduction systems. It is noteworthy that the majority of GPCR-targeted drugs elicit their biological activity by selective agonism or antagonism of biogenic monoamine receptors, while the development status of peptide-binding GPCR-adressing compounds is still in its infancy.

Exemplified on selected medicinal chemistry projects, this review will focus on the opportunities of therapeutic intervention into a broad spectrum of disease processes through agonizing or antagonizing the functions of peptide-binding GPCRs. In this context, a brief overview of GPCR-mediated signal transduction pathways will be given in order to emphasize the biomedical relevance of a controlled modulation of receptor function. Modern trends on lead finding and optimization strategies for peptide-binding GPCR-targeted low-molecular weight compounds will be highlighted on the basis of current research programs conducted in the areas of angiotensin II, endothelin, bradykinin, neurokinin, neuropeptide Y, LHRH, C5a antagonists, and somatostatin agonists, respectively. Special emphasis will be laid on the elaboration and utilization of structural rationales on the potential drug candidates, thus facilitating more detailed insights into the underlying molecular recognition event.

INTRODUCTION

Current pharmaceutical research is going through a period of unprecedented change, since new revolutionizing techniques have been successfully implemented into the pharmaceutical discovery process. At the same time, pharmaceutical industry feels growing pressure to release more new chemical entities (NCEs) that evolve as highly selective drugs targeting therapeutic areas of unmet medical need and address novel mechanisms of action. These attributes clearly define an ideal set of preconditions for positioning a candidate with block buster potential onto the drug market [1-3]. The conceptual combination of automated combinatorial chemistry, multiple parallel synthesis with high-throughput screening has dramatically altered the process of lead finding in medicinal chemistry in that vast numbers of low molecular weight compounds can rapidly be screened against biological target systems [4]. This progress in medicinal chemistry is paralleled on the side of target identification and validation with the maturation of genomics, proteomics, and bioinformatics in pharmaceutical research [5]. Taken together, these novel methodologies are expected to facilitate and accelerate the overall drug discovery process significantly.

However, the judicious choice of a disease relevant target is still one of the most crucial steps in initiating a drug discovery project, both in terms of novelty and uniqueness of the underlying therapeutic principle, as well as the competitor situation [2].

In this context, the superfamily of transmembrane G protein-coupled receptors (GPCRs) emerged as the most prominent class of qualified drug targets for pharmaceutical research and biomedical application [6]. Approximately 60% of all commercially available drugs work by selective modulation of distinct members of this target family [7]. Even though an estimated number of 1000 to 2000 GPCRs is expected to exist in the human genome [8], current GPCR-targeted therapeutic principles exploit a surprisingly small fraction of the GPCR family known today. A strong bias exists among the GPCR-targeted drugs in favour of the subclass of biogenic monoamine-stimulated GPCRs, i.e. the classical neurotransmitter-binding receptors [9,10].

This review will focus on the opportunity to further expand the spectrum of drug-targeted GPCRs onto the huge subclass of peptide-binding representatives of that target family. After a brief introduction on the basic principles of receptor structure and function, the chemically diverse set of endogenous ligands will be discussed with the aim to emphasize the relevance of peptide-binding GPCRs for modern drug discovery.

The lead identification and optimization attempts discussed in this contribution are restricted on projects that are aimed to identify peptidomimetic or non-peptide agonists or antagonists. Numerous pharmaceutical research efforts conducted over the last two decades have clearly proven the

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relevance of an early pharmacokinetic profiling. Consequently, satisfactory metabolic stability and oral bioavailability demand a transfer of the peptide-encoded biological and structural information onto non-peptide, drug-like scaffolds in order to achieve the desired goal [11-13].

Classical attempts towards drugs selectively addressing peptide-binding GPCRs will be exemplified on the angiotensin II and endothelin receptor antagonists. In both areas, leads were identified by screening programs and further optimized by classical medicinal chemistry approaches to yield clinical candidates, some of which already entered the market. The classical approach of optimizing screening hits will further be introduced with medicinal chemistry programs aimed to identify active compounds for a modulation of the bradykinin, neurokinin, and NPY (neuropeptide Y) receptors. Since the area of peptide-binding GPCR compounds is still in its infancy, especially when compared to the situation of biogenic amine-binding receptor drugs, the actual state of the majority of projects discussed in this review is still in the preclinical or in early clinical phases. Apart from random lead finding attempts, structural rationales are more frequently used in recent times, precedented by studies on somatostatin, bradykinin, neurokinin, LHRH (luteinizing hormone-releasing hormone), and anaphylatoxin C5a receptor agonists and antagonists that will be discussed briefly. Structural rationales were mainly derived from an educated guess on the bioactive conformation of the endogenous peptide or protein ligand, thus offering the opportunity to follow an indirect drug design approach.

GPCR SUPERFAMILY

G protein-coupled receptors constitute the largest receptor family known today [8]. According to an analysis of the *C. elegans* genome [14], approximately 5% of the 19100 nematode genes encode GPCRs with a family distribution profile that is reminiscent to that of mammalian GPCR genes. Extrapolation of these findings would suggest that up to 5000 distinct GPCR-encoding genes exist within the human genome (5% of an estimated 100000 genes). Currently, more than 800 distinct members of the GPCR superfamily have been cloned from various species, ranging from fungi over plants, yeast, slime mould, protozoa, metazoa to humans. Apart from the sensory olfactory receptors, approximately 150 human GPCRs have been cloned for which also the endogenous ligands have been identified. Further, more than 100 GPCRs are known with unidentified ligands and unknown physiological relevance, so called orphan GPCRs, which undoubtedly represent a rich source of disease-relevant drug targets for future biomedical research [15-17].

Structure and Function of GPCRs

GPCRs belong to the class of integral plasma membrane proteins and share a common receptor protein topology throughout the entire family. The structure paradigm is a seven helix bundle that spans the cell membrane in an almost perpendicular orientation, thereby establishing a functional link between the exterior and the cytoplasm of the

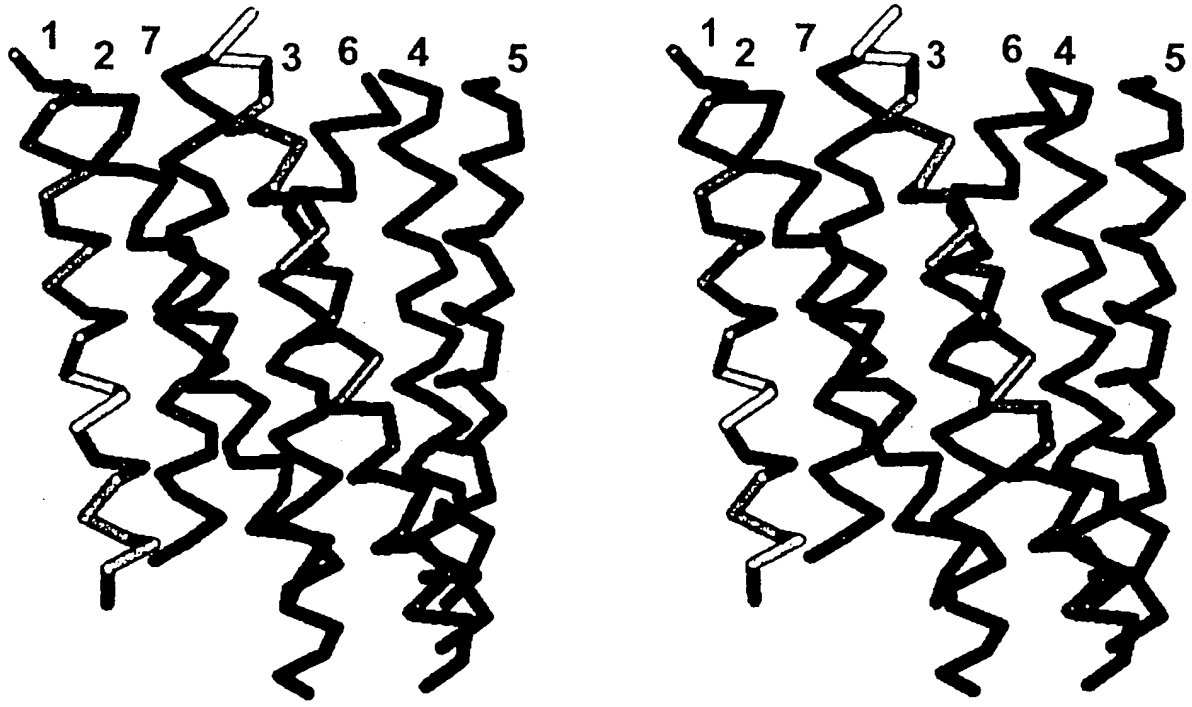


Fig. (1). Side-by-side stereo presentation of the Cα trace model of rhodopsin derived from various biophysical and bioinformatics studies. The helix bundle is shown in a sideview, the extracellular compartment being on the top. For details see references [22-31].

cell [6,18-20]. The seven transmembrane sequence stretches can be identified by hydrophobicity analyses since they exhibit an increased hydrophobic signature in a corresponding hydrophobicity profile. From numerous biophysical and biochemical studies it is now general accepted that GPCRs intercalate into the cell membrane with their *N*-terminus in the extracellular compartment, while the *C*-terminus reaches into the cytoplasm of the cell. The seven transmembrane helices (7TM domain) that constitute the central core domain of all GPCRs, are sequentially connected by extracellular and intracellular loops. Apart from variations in the primary structure, GPCRs differ in length of these loops, as well as in length and function of both *N*- and *C*-termini. The ACTH (adrenocorticotropic hormone) receptor is one of the smallest GPCRs known with 297 residues. Biogenic monoamine receptor sequences cover a size from approximately 350 to 600 residues, peptide receptor sequences are found between 400 and 750 residues, while the mGluRs (metabotropic glutamate receptor) mark the upper boundary consisting of roughly 1200 amino acid residues [21].

Even though no high-resolution structure of any pharmaceutical relevant member of the GPCR superfamily has been determined by e.g. x-ray crystallography, low resolution models derived from electron cryo-microscopy and electron diffraction of bovine, frog and squid rhodopsin reveal a detailed picture of the insertion mode of each helix within the context of the transmembrane helix bundle domain (Fig. (1)) [22-31].

From a functional point of view, GPCRs share a common property in that they work as transmembrane transducer systems by transferring an extracellular message across the cell membrane, thus allowing the affected tissue to respond to a broad range of signalling molecules [32-35]. Upon extracellular binding of the molecular stimulus, the central core domain (7TM domain) is believed to undergo a conformational change, thereby transmitting the extracellular binding event into the cytoplasm (Fig. (2)). The binding of a receptor agonist leads to an intracellular interaction of the receptor protein with its cognate heterotrimeric GDP-bound G protein. The agonist-promoted conformational change of the receptor protein followed by the cytoplasmic G protein-coupling initiates the activation of intracellular effector systems by the G protein cycle (Fig. (2)). The coupling event catalyzes the exchange of GDP against GTP and the dissociation of the GTP-bound α subunit from the $\beta\gamma$ heterodimer. Depending on the very nature of the G protein α subtype, different effector systems such as enzymes (e.g. adenylyl cyclase, phospholipase C) or ion channels are functionally modulated, which substantially amplifies the production of second messengers. The effector activation event is accompanied by a GTPase activity of the α subunit releasing inorganic phosphate. The GDP-bound form converts the α subunit to exhibit high affinity for the $\beta\gamma$ heterodimer, finally forming the GDP-bound heterotrimeric G protein again. The modulated concentration of second messengers elicits phosphorylation cascades across the cytoplasm to the nucleus, eventually activating the final physiological response of a cell to the original extracellular stimulus. Even though this functional paradigm accounts for all known GPCRs, this obvious convergence after the ligand binding event is diversified by the selective activation of only distinct types of G proteins from which e.g. numerous different G_α subunits are known (Fig. (2)) [32-35].

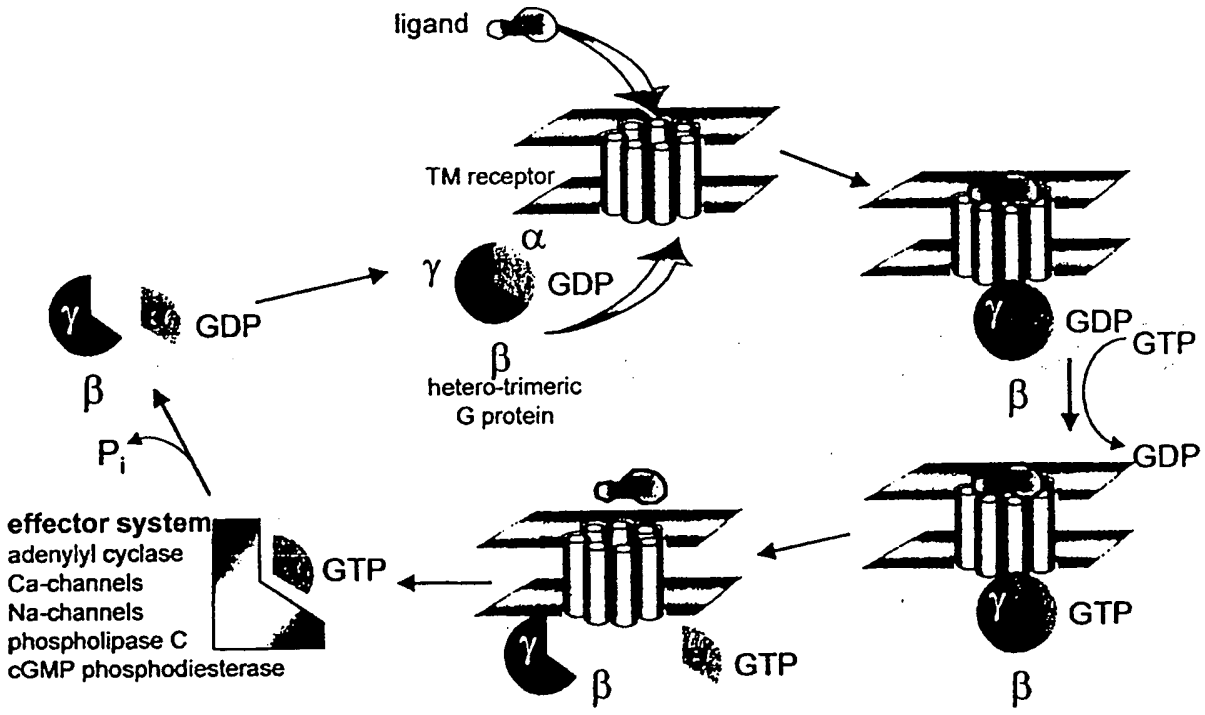


Fig. (2). Schematic representation of the ligand-GPCR interaction mediated G protein cycle.

In order to fully characterize the mechanism of action of GPCRs, a thermodynamic "eight-state-model" has been developed as a mechanistic hypothesis describing the macroscopic properties of transitions among distinct conformational states (Fig. (3)) [36]. The simplest way to describe the ligand-induced receptor activation event is a ternary complex model (A) that defines two distinct affinity states of the receptor for agonist binding, notably the free receptor (*Rec*) and the G protein-bound form (*G•Rec*) (Fig. (3)A). Agonists would display high affinity to the G protein-associated receptor, while antagonists would exhibit only low-affinity towards the complex. With the discovery that GPCRs can activate G proteins in the absence of any agonist, the simple ternary complex model required an extension. To account for the existence of such constitutively active GPCRs, a receptor activation step in the unliganded form was introduced (Fig. (3)B). This receptor isomerization hypothesis resulted in a "six-state-model" in which the activated receptor (*Rec**) is capable of signalling in both the G protein-associated form (*G•Rec**), and in the ternary complex (*G•Rec*•Lig*). The problem with that receptor activation-extended ternary complex model is that the G protein only binds to the receptor in its activated form *Rec**. Experimental evidence clearly suggests that G proteins do also bind to the resting state (*Rec*) without subsequent G protein activation. To account for these findings and to refer to the microscopic reversibility principle of thermodynamics, an "eight-state-model" was proposed in which the receptor protein can undergo three distinct processes, namely (i) ligand binding, (ii) receptor isomerization, and (iii) G protein binding (Fig. (3)C). Agonists can bind to four different receptor states clearly favouring the activated states generated either by receptor isomerization or by G protein association. Inverse agonists would prefer to bind the non-activated groundstate (*Rec*), while partial agonists show affinity to both receptor states but still cause receptor activation. In the thermodynamic "eight-state-model" an antagonist would just block the interconversion of different states rather than preferably bind to distinct states (Fig. (3)) [36].

In order to address phenomena such as isosteric or allosteric antagonism, structural models with atomic resolution are mandatory that are actually frequently used for both rationalizing structure-activity relationships of low molecular weight agonists and antagonists, as well as understanding the results from site-directed mutagenesis experiments. A detailed discussion of the actual status of experimentally derived, and molecular modeling derived GPCR structures [37] is beyond the scope of this review, since this contribution is mainly aimed to introduce the currently applied technologies to identify compounds selectively modulating peptide-binding GPCRs.

GPCR Classification

Exhaustive sequence analysis revealed three major homology families for the mammalian GPCRs, notably the family 1 or rho-family (prototype: rhodopsin), the family 2 or scr-family (prototype: secretin receptor), and the family 3 or mGluR family (prototype: metabotropic glutamate receptors) receptors (Fig. (4)) [32-35]. Family 1 receptors are divided into further subfamilies according to the size and

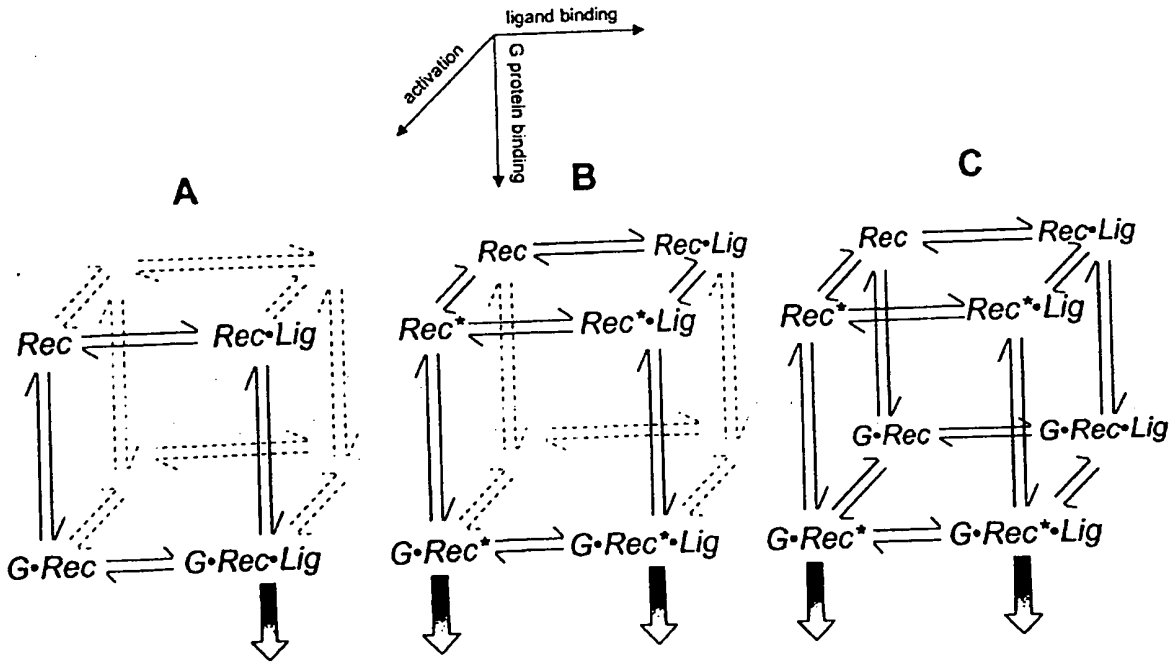


Fig (3). Mechanistic characterization of GPCR activation; A: "four-state" model; B: "six-state" model; C: "eight-state" model. Rec: receptor protein; Rec*: activated receptor protein; G: G protein; Lig: ligand molecule; filled arrows mark complexes capable of signalling (for details see text).

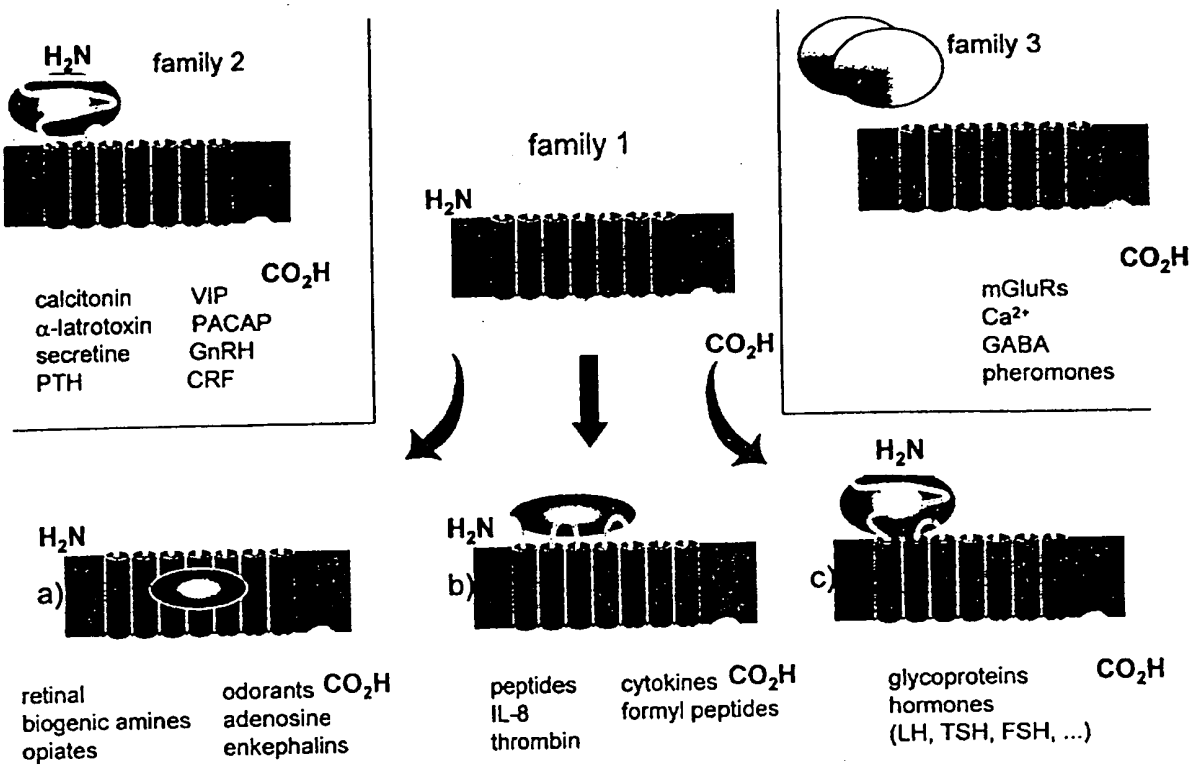


Fig. (4). Sequence homology-derived classification of GPCRs. Each GPCR family is characterized by a common ligand binding mode.

chemical nature of their corresponding agonists, as well as the mode of ligand binding. Family 1a accommodates the β-adrenoceptor-type receptors that are activated by small ligands such as biogenic monoamines, opiates, nucleotides, and small peptides, that comparably bind to a transmembrane cavity formed by helices 3, 4, 5, and 6. Family 1b is composed of receptors stimulated by oligopeptides and proteins such as IL-8 (interleukin-8), cytokines, and thrombin. The ligand binding epitope is located in the extracellular loop region. Family 1c receptors recognize glycoprotein hormones such as LH (luteinizing hormone), TSH (thyroid-stimulating hormone), and FSH (follicle-stimulating hormone) while their ligand binding site is centred in a large extracellular *N*-terminal domain (Fig. (4)).

Family 2 receptors are distinct from rho-family receptors in that they bind large peptides like glucagon, secretin, PTH (parathyroid hormone), VIP (vasointestinal peptide), or CRF (corticotropin-releasing factor). Comparable to family 1c receptors, the secretin family utilizes a large *N*-terminal domain for ligand binding. Family 3 receptors are unique since they possess a large extracellular *N*-terminal domain of several hundred residues that constitutes the binding site for smallish ligands such as a single divalent Ca^{2+} cation, glutamate, GABA (γ-amino butyric acid), and pheromones (Fig. (4)).

On the light of this classification, peptide-binding receptors are not structurally homogenous since they belong to family 1 and 2. Consequently, correlation of sequence homology with ligand similarity remains questionable which is also reflected by the mutual different binding modes of peptidic and non-peptidic agonists and antagonists.

Ligand Variety

GPCRs are stimulated by an amazingly large number of agonists covering a broad range of chemical diversity. Ligands are as small as divalent cations, biogenic monoamines such as acetylcholine or serotonin, fragrances and taste molecules such as aspartam or limonen, single amino acids such as glutamate or GABA, or nucleotide analogues such as adenosine. Medium-sized ligands range from cannabinoids over prostaglandines to small oligopeptides such as enkephalins, angiotensin II, bradykinin, somatostatin, and tachykinins. Larger oligopeptides and globular proteins constitute the family of macromolecular ligands including e.g. neuropeptide Y, C5a anaphylatoxin, interleukin-8, or chemokines. Even proteolytic enzymes such as thrombin, which activates its receptor by cleaving off an *N*-terminal peptide, selectively bind to distinct members of the GPCR superfamily. Apart from their important role in sensory perception including

vision, smell, and taste, GPCRs are obviously optimized by Nature for recognition and transduction of messages from different compound classes, i.e. nucleosides, lipid mediators, neurotransmitter, peptides, and proteins [6,18,38].

In this context, it is interesting to note that the majority of GPCR-targeted therapeutic principles exploit only a single compound class, notably the neurotransmitters. When the number of currently identified neurotransmitter receptors is compared with the number of disease-relevant peptide-binding GPCRs, an obvious imbalance becomes apparent in that only a small number of peptide-binding GPCRs is targeted by established therapies. Agonism and antagonism of e.g. α and β adrenoceptors, dopamine, histamine, serotonin, or muscarinic acetylcholine receptors are well established therapeutic principles for numerous "best-selling" drugs covering virtually all therapeutic areas, including gastrointestinal, cardiovascular, and CNS indications. In contrast, only two peptide-binding GPCR families are addressed by marketed non-peptide drugs, namely the opioid receptors and the angiotensin II receptor. However, the importance of peptide- and protein-binding GPCRs for drug discovery continues to be manifested by the fact that across current pharmaceutical research, especially in industry, numerous projects are pursued to identify leads that, upon optimizations fulfil all pharmacodynamic and pharmacokinetic demands required for clinical applicability (Table 1).

CLASSICAL LEAD FINDING AND DRUG DEVELOPMENT

Currently applied drug design and discovery approaches are typically classified as rational or random, depending on whether or not structural rationales are employed. The area of GPCR agonists and antagonists research is mainly driven by screening approaches in which large numbers of randomly selected chemical entities are tested in high-throughput screens. These shotgun procedures provide a practical means for identifying new leads for a particular receptor. In the following, this classical approach for GPCR-targeted drug discovery will be exemplified with prototype studies conducted on the angiotensin II, endothelin, bradykinin, neurokinin, and NPY receptors, respectively.

Table 1. Selection of endogenous Peptides that Exert their Biological Activity by Selective Activation of a GPCR

GPCR	code	native ligand (peptide/protein)	nature of the ligand
angiotensin receptors	AT_1, AT_2	angiotensin II (AII)	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
bombesin receptors	BB1 - BB4	bombesin, neuromedin B, gastrin-releasing peptide	14 aa peptide amide
bradykinin receptors	B_1, B_2	bradykinin (BK)	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
C3a receptor	C3aR	C3a anaphylatoxin	protein
C5a receptor	C5aR	C5a anaphylatoxin	protein
CC chemokine receptors	CCR1 - CCR9	chemokines	proteins
CXC chemokine receptors	CXCR1 - CXCR5	chemokines	proteins
cholecystokinin/gastrin receptors	CCK_A, CCK_B	cholecystokinin (CCK), gastrin	33 aa peptide amide, 17 aa peptide amide
endothelin receptors	ET_A, ET_B	endothelin-1 (ET-1), ET-2, ET-3	21 aa peptides
alpha factor pheromone receptor	STE2, STE3	fungal mating pheromones	13 aa peptide
fMet-Leu-Phe receptor	fMLP-R	Formylpeptide (fMLP)	FMet-Leu-Phe
galanin	GAL1, gal2, gal3	galanin	30 aa peptide
melanocortin receptors & ACTH receptor	MC_1, MC_3, MC_4, MC_5 MC_2 = ACTH receptor	melanocortin (MSH) adrenocorticotropic hormone (ACTH), corticotropin	39 aa peptide
neuropeptide Y receptor	Y_1 - Y_6	neuropeptide Y (NPY), peptide YY (PYY), pancreatic polypeptide (PP)	36 aa peptide amide (NPY)
neurotensin receptor	NTS1, nts2	neurotensin	13 aa peptide
opioid receptors	δ	[Met]-enkephalin, [Leu]-enkephalin	Tyr-Gly-Gly-Phe-Met/Leu
	κ	dynorphin A	17 aa peptide
	μ	β-endorphin, Lipotropin C fragment	31 aa peptide
nociceptin receptor	ORL1	nociceptin, orphanin FQ	17 aa peptide
somatostatin receptors	sst_1 - sst_5	somatostatin	cyclic14 aa peptide
tachykinin receptors	NK_1	substance P	11 aa peptide
	NK_2	neurokinin A (NKA), substance K, neuromedin L	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
	NK_3	neurokinin B (NKB), neuromedin K	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2
thrombin / protease-activated receptors	PAR1, PAR2, PAR3, PAR4	thrombin, trypsin, factor Xa	protein

(Table 1). contd.....

GPCR	code	native ligand (peptide/protein)	nature of the ligand
vasopressin receptors	V_{1A}, V_{1B}, V_2	vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2
oxytocin receptor	OT	oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
vasotocin receptor	VT	vasotocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly- NH_2
orexin receptors	OX_1, OX_2	orexin A/B	33 aa/28 aa peptide amides
FSH receptor	FSH receptor	follicle-stimulating hormone (FSH)	protein
LSH receptor	LSH receptor	lutropin, choriogonadotropic hormone, lutenizing hormone	protein
TSH receptor	TSH receptor	thyrotropin, thyroid-stimulating hormone	protein
LHRH receptor	LHRH receptor	gonadotropin-releasing hormone (GnRH), luteinizing hormone-releasing hormone (LHRH)	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly- NH_2
thyrotropin-releasing hormone & secretagogue receptors	TRH_1, trh_2	thyrotropin-releasing hormone/factor (TRH/F)	pGlu-His-Pro-NH_2
GHS receptor	$GHSR_{1a}$, $GHSR_{1b}$	growth hormone secretagogues (GHS)	oligopeptides
calcitonin/calcitonin gene-related peptide receptors	CGRPR	calcitonin, calcitonin gene-related peptide (CGRP)	32 aa peptide amide
amylin receptor	amylin receptor	amylin	37 aa peptide amide
adrenomedullin receptor	adrenomedullin receptor	adrenomedullin	52 aa peptide amide
corticotropin-releasing factor receptor	CRF_1, CRF_2	corticotropin-releasing factor (CRF)	41 aa peptide amide
gastric inhibitory peptide receptor	gip receptor	gastric inhibitory peptide (GIP)	42 aa peptide
glucagon/glucagon-like peptide receptor	GLP1	glucagon	29 aa peptide
growth hormone-releasing hormone receptor	GHRH receptor	growth hormone-releasing hormone/factor (GHRH/GRF)	44 aa peptide amide
parathyroid hormone receptor	type 1, type 2	parathyroid hormone (PTH)	84 aa peptide
secretin receptor	secretin receptor	secretin	27 aa peptide amide
vasoactive intestinal peptide & PACAP receptor	$VPAC_1$, $VPAC_2$, PAC_1	vasoactive intestinal peptide (VIP) pituitary adenylate cyclase activating peptide (PACAP)	28 aa peptide amide 38 aa peptide

Angiotensin-II Antagonists

Biomedical Significance

The endogenous octapeptide hormone angiotensin-II (A-II) (Table 1), Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, is the key effector compound of the renin-angiotensin system (RAS) which is one of the main blood pressure and electrolyte/fluid homeostasis regulating system in mammals [39]. As a result of a proteolytic cascade starting with angiotensinogen, angiotensin-II is released from its precursor decapeptide angiotensin-I by the action of angiotensin-I converting enzyme (ACE), the latter being a qualified target of antihypertensive drugs [40]. The conversion from angiotensinogen to angiotesin I is catalyzed by the aspartic protease renin, peptide-type inhibitors of which have not yet reached an advanced state of clinical development [41]. A-II interacts specifically with two different receptor subtypes of the GPCR superfamily, notably the AT_1 and the AT_2 receptor, respectively [21]. Interaction with the AT_1 receptor causes severe vasoconstriction, aldosterone release, vasopressin secretion, and renal sodium reabsorption. These effects convergently result in a dramatic increase of extracellular fluid volume, thus giving rise for a significant hypertensive effect. Therapeutic intervention into the RAS clearly offers major clinical and commercial success as shown with the ACE inhibitors for the treatment of hypertension and congestive heart failure [40]. Due to the fact that ACE inhibitors cause dry cough and angioedema [42], new strategies have been sought to block the vasocontrictory activities of the biologically active player, A-II [43]. Specific inhibition of the A-II target receptor interaction, the final step of the RAS, offers an entirely new and selective approach to blocking this regulatory system regardless of the source of the biological active peptide. And indeed, selective nonpeptide A-II antagonists emerged as a new class of

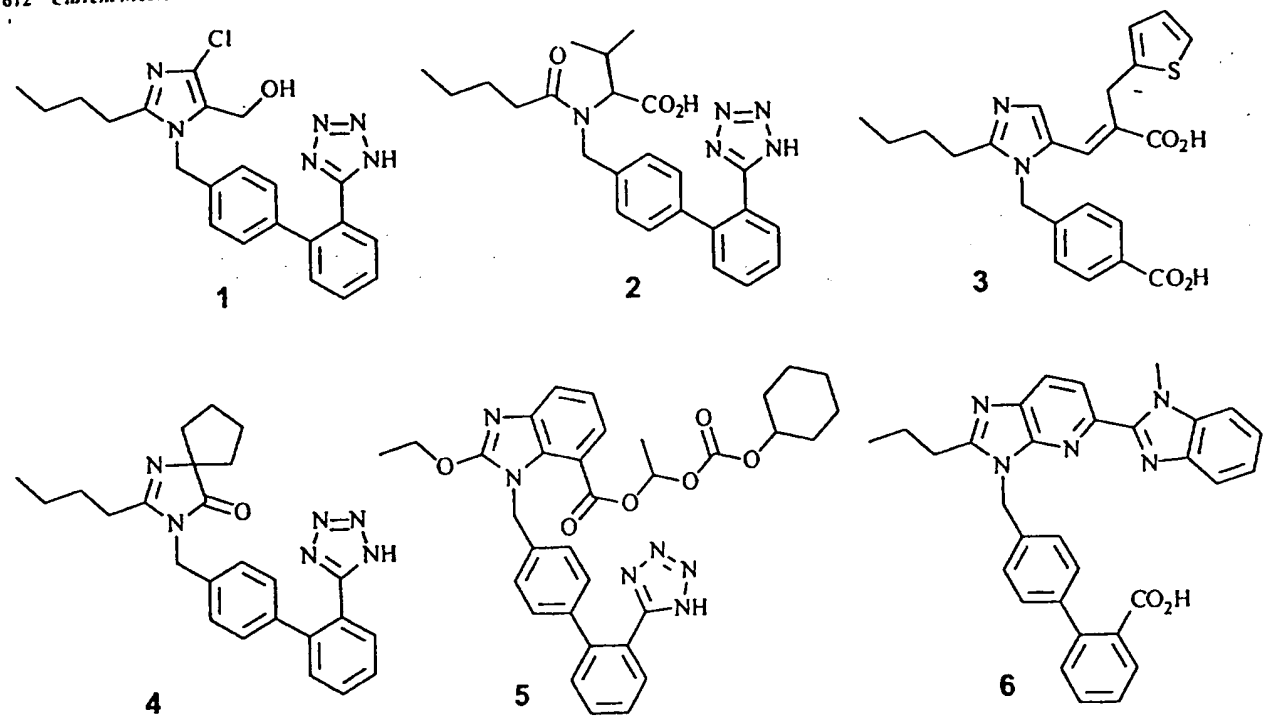


Fig. (5). Structures of marketed AII antagonists.

antihypertensives on the cardiovascular drug market exemplified by the released drugs Losartan **1** [44,45], Valsartan **2** [46], Eprosartan **3** [47], Irbesartan **4** [48], Candesartan **5** [49], and Telmisartan **6** [50], respectively (Fig. (5)).

Consequently, the angiotensin receptor represents one of the most advanced drug targets from the family of peptide-binding (non-opioid) GPCRs in the sense that screening hits have successfully been transferred to leads, further to development candidates that finally reached the drug market as save and innovative drugs introducing a new therapeutic principle.

Lead Finding

In the search for A-II antagonists potent peptides have been synthesized in a classical ligand-based design concept, yielding e.g. [Sar^1,Ala^8]-Angiotensin-II, commonly termed Saralasin [51]. However, all these peptides display limited therapeutic value as potential antihypertensives due to their poor oral bioavailability, rapid excretion, structural complexity, and significant agonistic profiles [51,52].

The feasibility of identifying nonpeptide AT receptor binding compounds with purely antagonistic profile was demonstrated by a research group at Takeda Chemical Industries in 1982. In a series of two patents, Furukawa and co-workers reported on the inhibition of angiotensin-II-induced contractile response in rabbit aorta by numerous different 1-benzylimidazole-5-acetic acid derivatives (Fig. (6)) [53]. The two compounds S-8307 **7** and S-8308 **8** mark the beginning of a new era of antihypertensive drug research in which almost any pharmaceutical company attempted to derive new compounds from that initial findings.

Drug Development

The Takeda compounds served as lead structures for the development of highly potent and selective analogues at DuPont that culminated in Losartan **1** (DuP-753, EXP-7711), the first nonpeptide A-II antagonist that got approval by the FDA and reached the market (Fig. (7)). Guided my molecular modeling studies, the substitution pattern of the benzylic phenyl-ring was changed yielding EXP-6155, **9** which displayed a ten-fold increased binding affinity over e.g. S-8307 **7** [54]. Further extension in *para*-position of the

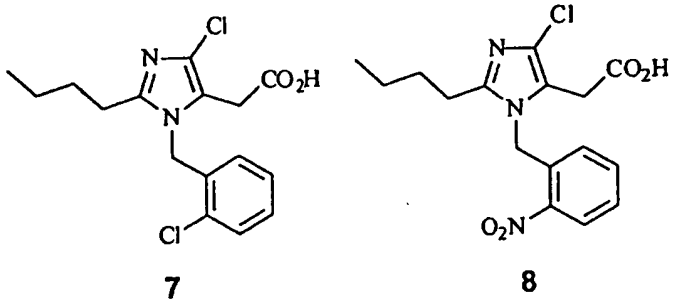


Fig. (6). Initial lead structures disclosed by Takeda Chemical Industries.

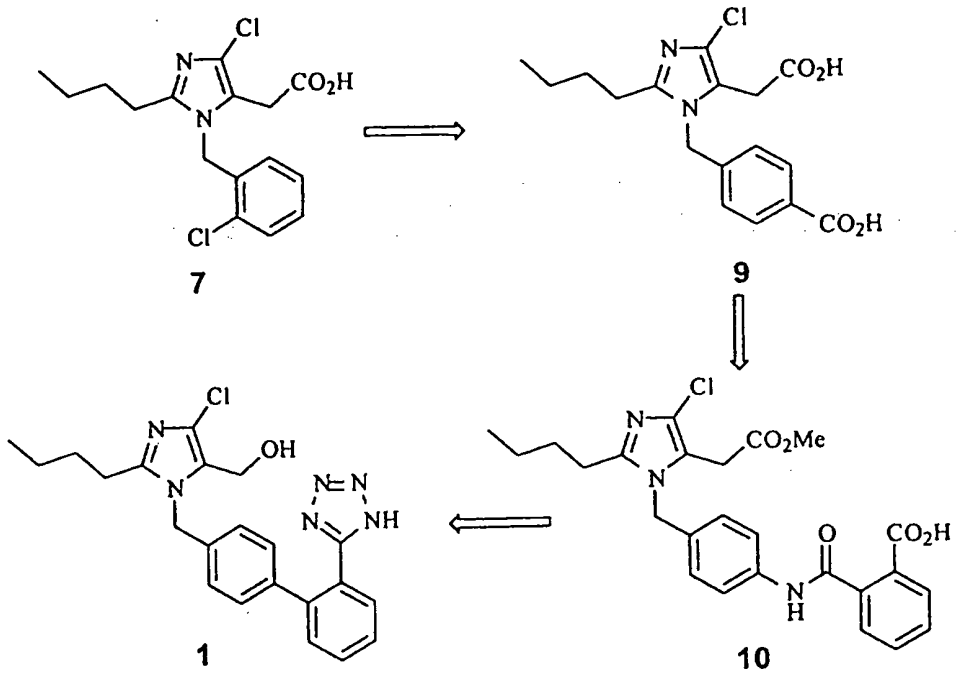


Fig. (7). Development of Losartan 1.

aromatic ring resulted in more potent analogues as shown with EXP-6803 10 [55].

The deletion of the interaromatic carboxamide linkage yielding biphenylmethyl-substituted imidazole-5-acetic acid derivatives produced orally active compounds and subsequent exchange of the *ortho*-carboxylic acid on the terminal aromatic ring against the tetrazole moiety further improved the oral activity [56,57]. The imidazole-5-acetic acid substituent was modified to the corresponding alcohol in the analogue chosen as clinical candidate. However, later it could be shown that the parent acetic acid sidechain of the imidazole core is the active metabolite of Losartan 1 [58].

Instead of modifying the N-1 substituent of the Takeda imidazole derivatives, 7 and 8, SmithKline Beecham decided to explore the 5 position in more detail (Fig. (8)). Introduction of an acrylic acid in that position (11) resulted in a 15-fold enhancement in binding affinity. Further introduction of a 2-thienylmethyl group in α-position of the

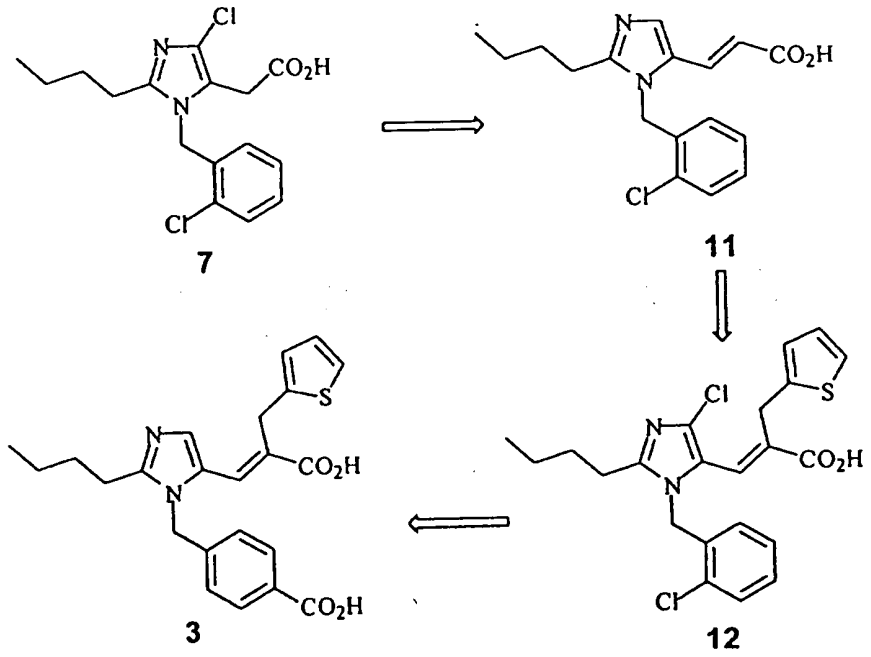


Fig. (8). Development of Eprosartan 3.

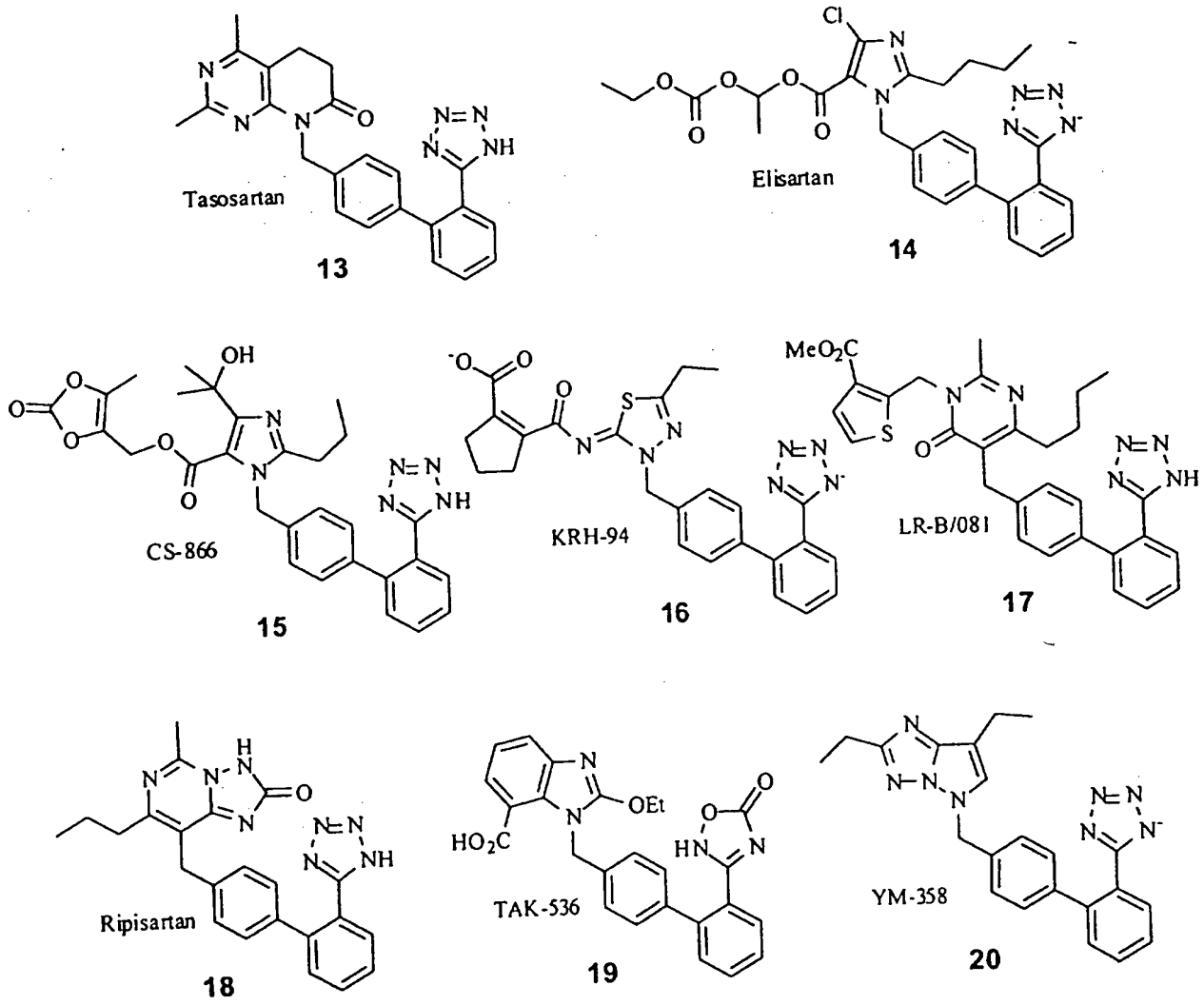


Fig. (9). Next-generation "sartans" in advanced states of clinical development.

acrylic acid substituent (12) together with a modification in the N-1 benzylic substituent finally yielded SK&F-108566, 3 [59,60] which inhibits A-II binding to its receptor in the single digit nanomolar range [61].

The Ciba compound CGP-48933, 2 (Fig. (5)) is the result of an optimization process attempting to replace the imidazole ring structure originally described by Takeda [53]. The 1-benzyl-2-butyl-4-chloro-imidazole-5-acetic acid is replaced with an *N*-terminally acylated amino acid, notably valine. CGP-48933, 2 has passed the clinical development and reached the market as Valsartan [62]. It is clearly beyond the scope of this review to systematically summarize the lead optimization programs pursued by the different pharmaceutical companies, however, it should be emphasized that, apart from the currently marketed drugs, numerous next-generation compounds and follow-ups in late clinical development are expected to get approved in the near future (Fig. (9)) [63,64]. These new "sartans" (13 - 20) together with the first generation drugs (1 - 6) will further change the landscape of antihypertensive prescription drugs since they clearly introduced a new quality of antihypertensive principles into therapy of cardiovascular diseases.

Apart from these biomedical aspects, the development of the "sartans" acting specifically on a member of the GPCR superfamily evolved to a textbook example of protein-targeted drug design within modern medicinal chemistry [65].

Endothelin

Biomedical Significance

Endothelin 1 (ET-1) is a 21 amino acid bicyclic peptide (Table 1) that was initially isolated from porcine aortic endothelial cells [66]. The endothelins constitute a class of three related isopeptides (ET-1, ET-2, ET-3) [67], exhibiting vasoconstrictive and mitogenic potential [68] upon binding to two receptor subtypes, notably the ET_A and ET_B receptor [69,70]. ET-1 selectively binds to the ET_A receptor which is expressed on vascular smooth muscle cells

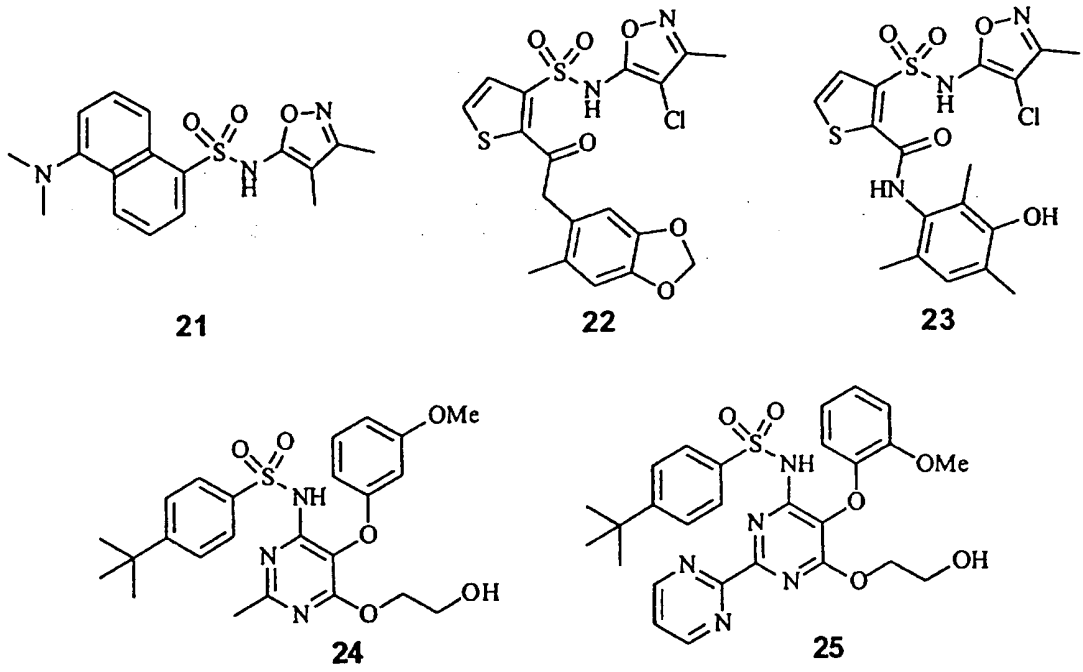


Fig. (10). Aryl-sulfonamide-type ET antagonists.

(lung, aortic, heart) and mediates vasoconstriction and proliferation through activation of a complex intracellular signalling cascade [71,72]. The ET_B receptor, localized in the brain, on vascular endothelial cells, and smooth muscle cells, is responsible for vasodilation via the release of nitric oxide, prostacyclin, and adrenomedullin [73,74]. In addition, ET_B functions as a clearance receptor for endogenous ET by the internalization of the receptor-ligand complex. On the other hand, ET_B may also cause vasoconstriction in some tissues [75]. ET_A and ET_B receptors share high sequence similarity (app. 68%). ET-1 is predominantly produced by endothelial cells acting in an autocrine and paracrine fashion as a mediator of vascular function. Elevated ET levels has been observed in tissue and plasma in a number of cardiovascular disorders, thereby contributing to disease states including hypertension [76], vasospasm, atheriosclerosis [77], acute myocardial infarction [78], congestive heart failure [79,80], restenosis [81], subarachnoid hemorrhage, ischemia, pulmonary hypertension [82], and renal failure [83]. Due to the pivotal pathophysiological role of the endothelin receptor-ligand interaction, this receptor system emerged as a promising target for therapeutic intervention in the disease states mentioned above [84].

Lead Finding

Since the discovery of ET-1 in 1988, a large number of potent antagonists have been described [84]. The first antagonists emerging from random screening efforts have been reported in 1992. These first generation compounds comprise anthraquinones from *Streptomyces misakiensis*, steroids isolated from bayberry, *Myrica cerifera*, and diphenyl ethers discovered in fungal broths [85]. Lead finding in this field is mainly based on compound library screening followed by classical lead optimization within medicinal chemistry programs. A number of peptide-based antagonists have been reported including the prominent cyclic pentapeptide BQ-123, and other peptide antagonists, e.g. BQ-788, FR-139317, PD145065, PD156252, RES-701-1, TAK-044, and IRL2500 [84-88].

As mentioned above, this review, will focus on the development of nonpeptide antagonists emerging from those programs directed towards the discovery of active low molecular weight compounds. Primarily, the ET_A-selective antagonists as well as antagonists exposing mixed ET_A/ET_B affinity play a major role for therapeutic intervention, even though some ET_B-selective antagonists have been reported only recently.

Aryl Sulfonamides

Bristol Myers Squibb designed BMS182874, **21**, a nonpeptide ET_A-selective antagonist from an initial hit which was discovered by screening of a sulfathiazole library [89]. The sulfonamide BMS182874, **21**, exhibits an IC_{50} value of 150 nM at the ET_A receptor (A10 cells) and shows no binding affinity to the ET_B receptor (Fig. (10)).

From a similar series of compounds, Immunopharmaceuticals (Texas Biotech.) developed an isoxaolyl-thiophene sulfonamide, TBC-11251, **22** (Sitaxsentan) [90]. This orally active compound has shown efficacy in phase II clinical trial of congestive heart failure (CHF) and demonstrated activity in a rat model of myocardial infarction and acute hypoxia-induced pulmonary hypertension (PH) [91]. Further investigations established a unique pharmacophore framework, characterized by a central thiophene subunit for selective ET_A antagonism [92]. Maintaining the sulfonamide substituent in position 3 and altering the substituent in position 2 in the thiophene ring

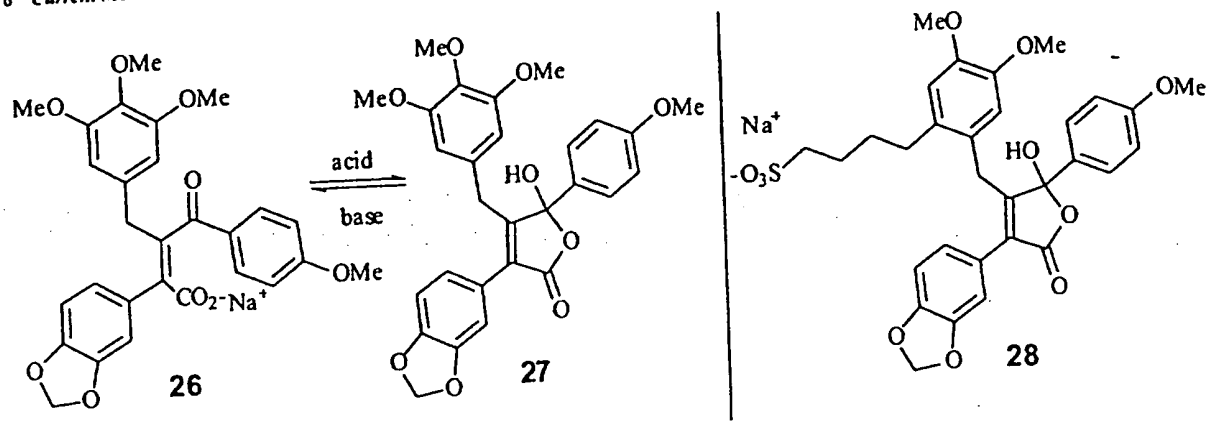


Fig. (11). Butenolide-type ET antagonists.

led to a series of compounds with enhanced pharmacological properties. TBC-2576, **23**, the optimal analogue in this series showed about 10-fold higher ET_A binding affinity compared to Sitaxsentan, **22**, and high ET_A-selectivity, as well as a serum half-life of 7.3 h in rats, paired with *in vivo* activity (Fig. (**10**)) [92].

A number of nonpeptide ET_A/ET_B antagonists based on a pyrimidyl-benzene sulfonamide scaffold have been reported. The first example for an orally active representative is Ro46-2005, **24** [93] which was obtained after optimization of a lead compounds identified by random screening in an antidiabetic project. The binding affinities of Ro46-2005, **24** (K_i=220 nM (ET_A), K_i=1000 nM (ET_B)) could further be optimized yielding the bipyrimidyl-benzene analogue Ro47-0203, **25** (Bosentan) which represents an improvement in both, receptor binding affinities (K_i=4.7 nM (ET_A), K_i=95 nM (ET_B)) and oral activity (Fig. (**10**)) [94]. Bosentan **25** is a competitive mixed ET_A/ET_B antagonist and shows promising results in clinical trials [88] in terms of vasodilation. Further, it improves left ventricular performance and reduces renal dysfunction. The beneficial effects of Bosentan **25** have been characterized in CHF models, in hypertension related experiments and in subarachnoid hemorrhage (SAH) trials. These and other potential applications have been described in a recent review by Roux *et al.* [88].

Butenolides

CI-1020, also known as PD156707, **26, 27** [95] emerged from the optimization of an initial lead structure which was identified from library screening (Fig. (**11**)). The optimization procedure was guided by following the *Topliss* "decision tree" approach based on QSAR principles [96]. CI-1020, **26, 27** represents the first clinical candidate emerging from the Parke-Davis series of butenolides. With an IC_{50} value of 0.30 nM on recombinant human ET_A receptor (IC_{50}=780 nM (ET_B)) it demonstrates high ET_A-selectivity (2600-fold). CI-1020, **26, 27** undergoes tautomerization, thereby establishing the γ-hydroxy butenolide structure **27** under acidic conditions, while at basic pH the equilibrium is shifted in favour of the ring-opened γ-keto acid salt structure **26** [95]. The poor water-solubility of this compound, caused by cyclization, has driven the drug development process towards a series of water-soluble ring-closed γ-hydroxy butenolides applicable for parenteral use [97]. One of the follow-up compounds exhibits promising pharmacological profiles by displaying improved activity compared to CI-1020, **26, 27** e.g. in preventing acute hypoxia-induced pulmonary hypertension (PH) in rats.

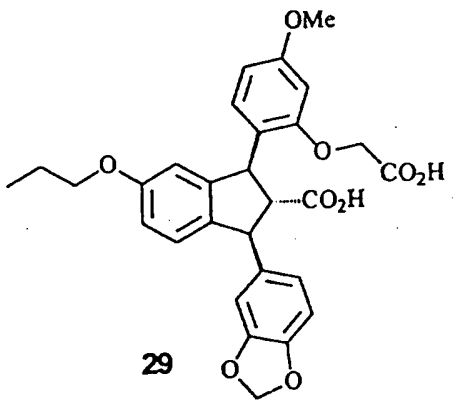
Most promising characteristics were found for an analogue containing the sodium salt of a sulfonic acid in compound **28** (Fig. (**11**)) [97]. It shows high ET_A-selectivity (4200-fold) with an IC_{50} value of 0.38 nM (ET_A) and ET_A functional activity of K_B=7.8, which is similar or even superior to the progenitor CI-1020 **26, 27**. Moreover, it displays improved water-solubility and shows higher activity after *i.v.* infusion in preventing acute hypoxia-induced PH in rats (ED_{50}=0.3 μg/kg/h) when compared to CI-1020 **26, 27** [97]. The new compounds are currently evaluated in preclinical trials, while CI-1020 **26, 27** has already been tested in a model of acute stroke and has entered clinical development for cerebral ischemia.

Indane Carboxylic Acids

SB209670 **29** emerged from the SmithKline Beecham laboratories after optimization of an initial hit discovered from compound library screening (Fig. (**12**)) [98]. Within a molecular modeling-driven approach based on a comparison of the NMR-derived conformation of ET-1 with the primary hit, an indene carboxylic acid derivative, the mixed ET_A/ET_B receptor antagonist SB209670 **29** was designed (K_i=0.43 nM (hET_A), K_i=14.7 nM (hET_B)). When administered *i.v.* SB209670 **29** shows efficacy in different animal models of ET-mediated disease states, e.g. renal failure, hypertension [84], and ischemia-induced stroke. Due to the low oral bioavailability (4%) a structurally related analogue, SB217242 **30** [99] was investigated that displays improved pharamacokinetics and bioavailability [86]. SB209670 **29** is under development (phase I) for acute *i.v.* indications with efficacy in pulmonary hypertension (PH), chronic renal failure (CRF) and stroke [87], while SB217242 **30** (phase I) is in development for chronic PH and chronic obstructive pulmonary disease (COPD) [87,100].

Pyrrolidine Carboxylic Acids

The SmithKline Beecham compound SB209670 **29** (Fig. (**12**)) served as template for the design of the



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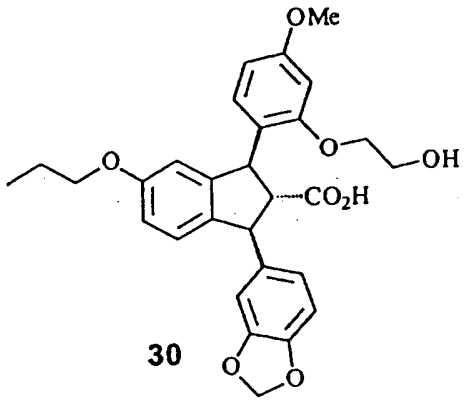

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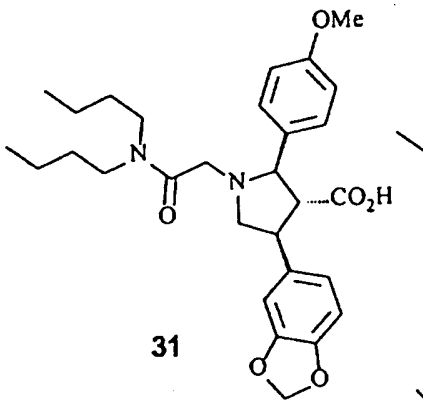
Fig. (12). Indane carboxylic acid-type ET antagonists.

pyrrolidine carboxylic acid A-127722 **rac-31** (Fig. (13)) [101], that has been disclosed as a potent, ET_A-selective antagonist, currently tested in clinical trials (PH, CHF) [87]. A-127722 **rac-31** was reported to prevent dose-dependently cerebral oedema in stroke-prone spontaneously hypertensive rats [100]. ABT-627 **31**, the active enantiomer (2*R*,3*R*,4*S*) of the *trans-trans* configurated 2,3,4-trisubstituted pyrrolidine ring, shows an IC_{50} value of 0.08 nM on ET_A and 8.1 nM on ET_B [102]. The 1800-fold selectivity was dramatically altered by subtle structural modifications of A-127722 **rac-31**, which led to A-182026 **32** with an ET_A/ET_B selectivity ratio of 3, thus being the most potent balanced dual ET_A/ET_B antagonist known today. Replacement of the dialkyl-acetamide (**rac-31**) against a 2,6-dialkyl-acetanilide resulted in an ET_B-selective antagonist, A-192621 **33** exhibiting promising pharamcological properties [103]. Combination of the structure-activity relationships (SAR) derived from the first series of ET_A-selective compounds (e.g. ABT-627 **31**) and the second series of ET_B-selective antagonists (e.g. A-192621 **33**) led to a further optimized series of compounds. Therein A-308165 **34** has been identified as highly selective (27000-fold), orally acitve ET_B antagonist [104].



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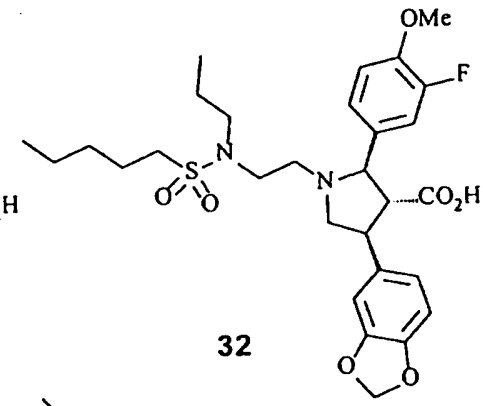

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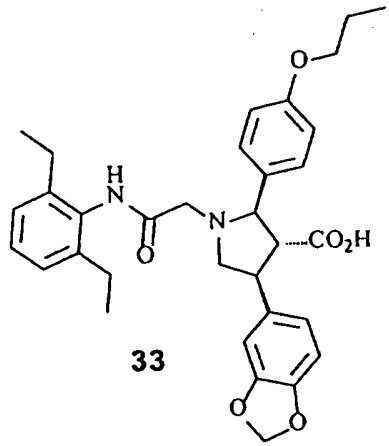

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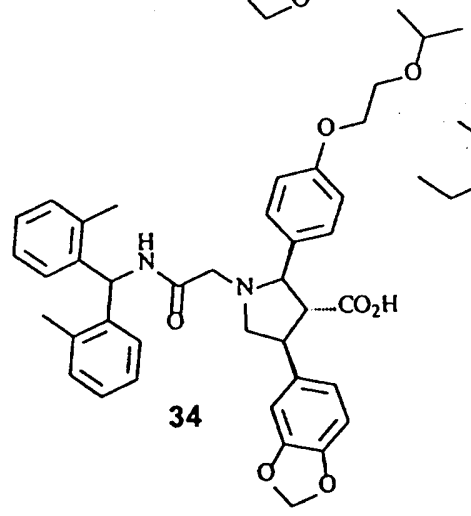

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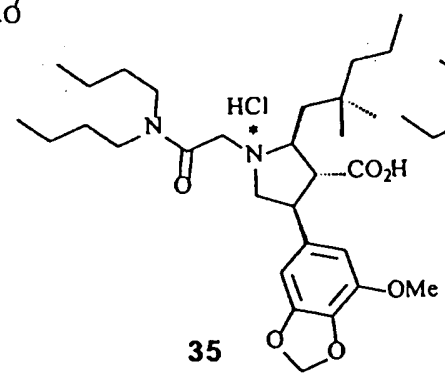

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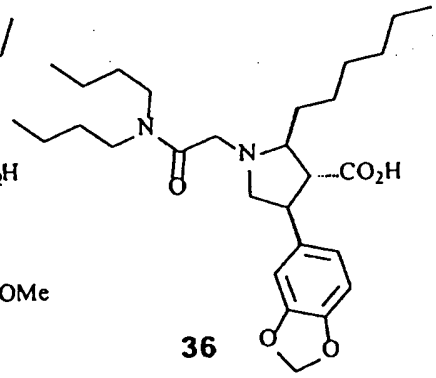

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Fig. (13). Pyrrolidine carboxylic acid-type ET antagonists.

Administration of ET_B-selective antagonist led to hypertensive responses indicating that they are not suitable as agents for a long-term systemic single ET_B-directed therapy [103]. Nevertheless, ET_B-selective antagonists are expected to be a valuable tool for the elucidation of the role of the ET_B receptor action under normal and pathophysiological conditions [104]. Most recently, an ET_A-selective antagonist, derived by optimization of A-127722 **rac-31**, emerged from the series of pyrrolidine-based compounds [105]. A-216546 **35** is a further orally active ET receptor antagonist showing >25000-fold selectivity for the ET_A receptor (K_i=0.46 nM), and is considered for clinical development as a therapeutic agent for chronic treatment of ET-1-mediated diseases [106]. Compound **36** (IC_{50}=5.6 nM (ET_A); >10000-fold selectivity) is currently under investigation at Abbott's Laboratories as ET_A antagonist. Apart from the ET receptor affinity, A-216546 **35** showed remarkable inhibition potential for numerous members of the GPCR superfamily such as adenosine receptors, δ-opioid receptor, purinergic receptor, etc. [106], thus indicating a kind of "ligand crosstalk" which turns out to be a common phenomenon of GPCR-targeted compounds.

Phenylacetamides

L-749,329 **37** (Fig. (**14**)) is an orally active, competitive and nonselective ET_A/ET_B antagonist developed by Merck inhibiting the binding of [^{125}I]ET-1 in Chinese Hamster Ovary (CHO) cells expressing human ET receptors with IC_{50} values of 0.8 nM (ET_A) and 16 nM (ET_B), respectively [107]. The active enantiomer, L-754,142 **37**, is a potent orally active ET antagonist with a long duration of action in several *in vivo* models. L-754,142 **37** shows binding affinity

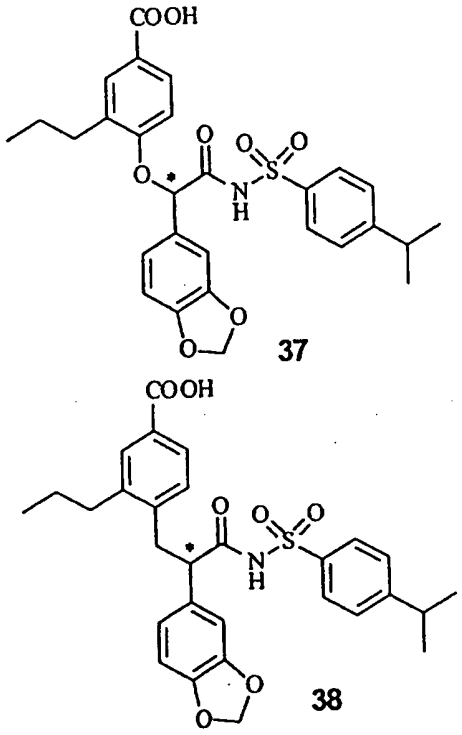


Fig. (14). Phenylacetamide-type ET antagonists.

towards ET_A (0.062 nM) and ET_B (2.25 nM) and antagonizes ET-1-induced phosphatidyl inositol hydrolysis in CHO cells expressing cloned human ET receeptors with IC_{50} values of 0.35 nM (ET_A) and 26 nM (ET_B) [108]. Substitution of the ether oxygen against a methylene group resulted in L-751,281 **38**, an analogue with similar activities on both ET receptor subtypes [107].

α-Phenoxyphenylacetic Acids

At the Merck laboratories, structural modifications of an initial lead discovered by screening for angiotensin II (AII) antagonists, led to a dual AT_1/ET antagonist. Further optimization towards ET_A-selectivity resulted in L-744,453 **39** (Fig. (**15**)), an α-phenoxyphenylacetic acid derivative lacking the sulfonamide present in the arylacylsulfonamides L-749,329 **37**, and L-751-281 **38** [107]. L-744,453 **39** competitively and reversibly inhibits [^{125}I]ET-1 binding to CHO cells expressing cloned human ET receptors with K_i values of 4.3 nM (ET_A), and 232 nM (ET_B). Thus, within L-744,453 **39** the shift from an originally angiotensin II antagonist to an ET-selective antagonist could be demonstrated, thus highlighting the potential of "cross-fertilization" of projects devoted to representatives of a common receptor superfamily.

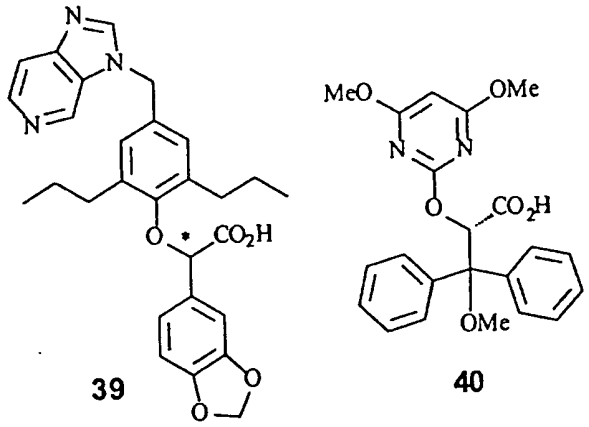


Fig. (15). Aryloxyacetic acid-type ET antagonists.

α-Aryloxyacetic Acids

Also at the BASF laboratories, the endothelin project started with screening of the in-house chemical substance stock. The initial lead, which was originally intended as a herbizide, was optimized by systematic structural modifications resulting in an ET_A-selective antagonist, LU135252 **40** (Fig. (**15**)), the active (*S*)-configurated enantiomer of LU127043 [109,110]. It selectively binds to the ET_A receptor with high affinity (K_i=2 nM (ET_A), K_i=184 nM (ET_B)) [111]. LU135252 **40** has been evaluated in clinical trials for preventing restenosis [87] and entered phase II for CHF [112]. Furthermore, it was demonstrated that selective ET_A receptor inhibition with LU135252 **40** could reduce ischemia-induced ventricular arrhythmias in pigs. Thus ET antagonism might reduce mortality by preventing arrhythmias, a major cause of death in CHF, obviously induced by the pro-arrhythmogenic effects of ET-1 [100].

Phenoxybutanoic Acids and Stilbene acids

According to a previously elaborated SAR study, Astles *et al.* at Rhône-Poulenc Rorer presented the optimized analogue RPR-111844 **41** (Fig. (16)), which exhibits an IC_{50} of 5.0 nM at the rat ET_A receptor and 1000-fold selectivity over the ET_B receptor. The promising pharmacokinetics in a rat model of ET-1-induced vasoconstriction rendered this RPR-111844 **41** an ideal candidate to examine these effects in preclinical models of cardiovascular disease [113].

In order to shed light on the characteristics of the bioactive conformation, a new series of rigidified analogues of stilbene acids were designed based on the SAR derived from a series of the phenoxybutanoic acids. Thus, compound RPR-111723 **42** was identified as the most potent analogue with an IC_{50} of 80 nM. Although the stilbene series was not further developed, results from SAR will be back-transferred into the more interesting series of phenoxy butanoic acids [114].

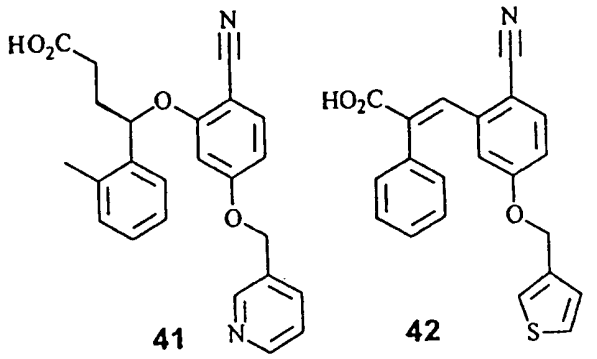


Fig. (16). Phenoxybutanoic acid- and stilbene acid-type ET antagonists.

Bradykinin

Biomedical Significance

The nonapeptide bradykinin (BK, Table 1), Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, belongs to the family of kinins. Kinins are small peptides which are released from kinninogens by several enzymes, the kallikreins [115-120]. Interaction of BK with two designated receptor subtypes, B_1 and B_2, results in a variety of biological effects including vasodilation, modulation of vascular permeability, smooth muscle contraction, recruitment and priming of inflammatory cells, induction of pain, modulation of transmitter release, stimulation of cell division, etc. [121]. Based on these diverse biological activities, BK is involved in inflammatory diseases, such as asthma, rhinitis, pancreatitis, sepsis, rheumatoid arthritis, brain oedema, and angioneurotic oedema [122]. Due to these pathophysiological actions of BK, mainly induced by the interaction with the B_2 receptor, this system emerged as an interesting target in pharmaceutical research. Hence, in a number of efforts BK antagonists were presented tempted to be a valuable tool in the treatment of above mentioned chronic diseases.

Lead Finding

'Second-Generation' B_2 Antagonists

Initiated by the discovery of NPC-567 by Vavrek and Stewart [123] in the 90's, a number of selective peptidic B_2 receptor anatgonists including Icatibant (Hoe-140) [124,125] and Bradycor (Deltibant, CP-0127) [126], so-called '*second-generation*' antagonists, have been clinically evaluated. In the following years, research programs were directed towards the discovery of B_2-selective nonpeptide antagonists. Detailed overviews on this subject were provided only recently by Altamura *et al.* [127] and Heitsch [128] addressing projects of diverse research group, and reviewing the current patent situation.

In 1993, the naphthylalanine derivative WIN-64338 **43** (Fig. (17)) was disclosed as the first nonpeptide B_2 antagonist [129,130]. A random screening approach at Sterling Winthrop led after optimization to compound WIN-64338 **43**, displaying a K_i value of 64 nM for the inhibition of [^{3}H]BK binding to the B_2 receptor (IMR-90 cells, fetal lung fibroblast cell line expressing the kinin B_2 receptor). However, this compound is problematic in terms of potency, oral bioavailability, and selectivity [130], since significant affinity for e.g. the muscarinic receptors was detected [131].

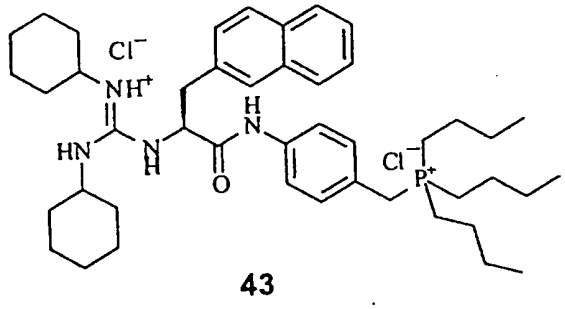


Fig. (17). 'Second-generation' B_2 antagonists WIN64338.

'Third-Generation' B_2 Antagonists

From 1994 on Fujisawa published a series of patent applications on new classes of potent, selective and orally active nonpeptide B_2 receptor antagonists [132-135], thereby establishing the so-called '*third-generation*' compounds. Several derivatives showed nanomolar affinity in receptor binding assays and high efficacy in various species including humans. They also exhibited *in vivo* functional antagonistic activity against BK-induced bronchoconstriction in guinea pigs and potency in diverse animal models of inflammation [132-135] [136,137]. Again, these compounds originally emerged from a random screening directed towards the angiotensin II (AII) AT_1 receptor and belong to a class of imidazo[1,2-*a*]pyridines. A detailed description of the design, synthesis and biological evaluation was given by Kayakiri *et al.*, only recently [138]. The first lead compound **44** (Fig. (18)) of this series of *N*-containing heteroaromatic benzyl ethers showed an IC_{50} value of 7.6 μM.

Within a classical medicinal chemistry approach based on SAR considerations the first lead compound **44** was exposed to extensive modifications leading to **45** (Fig. (18)).

This analogue displays an IC_{50} value of 2.4 nM for the inhibition of the specific binding of [^{3}H]BK to B_2 receptors in guinea pig ileum (GPI) membrane preparations. Thus, the 8-[3-(*N*-acylglycyl-*N*-methylamino)-2,6-dichlorobenzyloxy-3-halo-2-methylimidazo[1,2-*a*]pyridine skeleton was identified as the basic framework of the first orally active nonpeptide B_2 antagonist. In order to overcome species difference, further modifications within the 3-position of the benzyl moiety revealed an analogue (FR167344 **46**) exhibiting subnanomolar (IC_{50}=0.66 nM) and low nanomolar binding affinities (IC_{50}=1.4 nM) for GPI membrane and human A431 cells (epidermoid carcinoma cells) [136,139], respectively.

Recent results indicate that FR167344 **46** has specific antagonistic activity against guinea pig tracheal smooth muscle BK receptors, thus rendering it a potential therapeutic tool for the treatment of asthma [140]. Derivatives containing the *N*,*N*-dimethylcarbamoyl-substituted cinnamide group were capable of overcoming species differences, and therefore defined the required pharmacophore for further investigations. FR167344 **46** was assigned as new lead compound for three independent optimization approaches implying substitutions within the imidazo[1,2-*a*]pyridine moiety (benzimidazoles, quinoxalines, and quinolines). While further optimization of the quinoxaline series failed, optiization within the benzimidazole and quinoline series resulted in several potent congeners. Thus, consequent SAR studies of the benzimidazoles afforded improvements of *in vivo* oral activities, resulting in FR185627 **47** which exhibits 75.2 % inhibition against BK-induced bronchoconstriction at 0.32 mg/kg, *i.p.* [138]. Optimization of the quinoline series afforded compound FR173657 **48** with high potency in B_2 binding affinities for both GPI (IC_{50}=0.46 nM) and human recombinant B_2 receptors (IC_{50}=1.4 nM) [136,141]. FR173657 **48** displays the best *in vivo* B_2 antagonistic oral activity among nonpeptide antagonists investigated so far

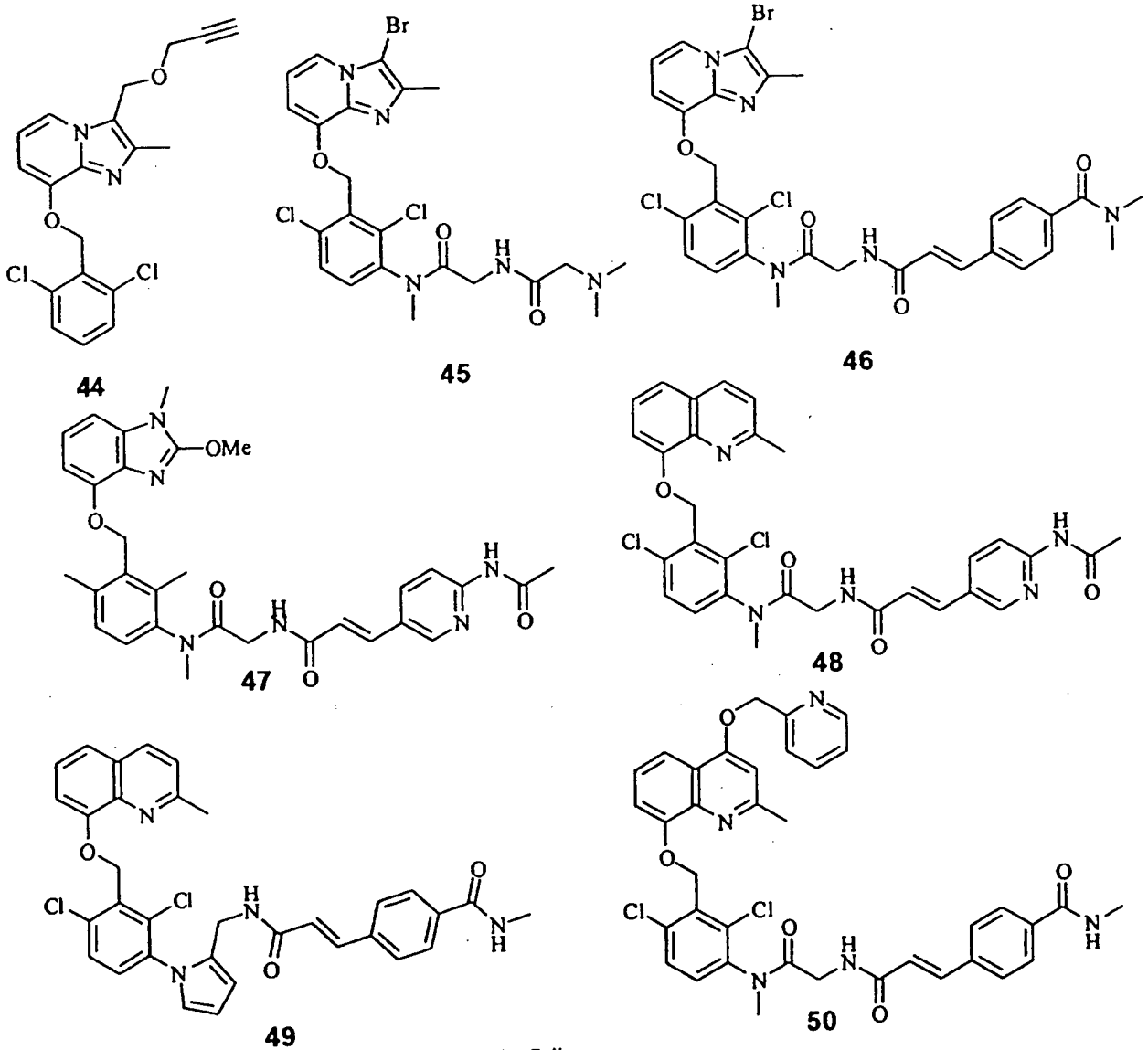


Fig. (18). 'Third-generation' B_2 antagonists developed by Fujisawa.

and was chosen as a clinical candidate for the treatment of various inflammatory diseases. Recent investigations on plasma extravasation mediated by activation of sensory nerves in guinea pig airways suggest FR173657 **48** to be an orally active, promising anti-inflammatory agent for kinin-dependent inflammation following antigen challenge [142]. Fujisawa researchers further report on the postulation of the active conformation of their compounds by synthezising conformationally restrained analogues. Molecular modelling studies and subsequent chemical synthesis of a novel pyrrole series afforded FR193144 **49**, an analogue which mimics the previously postulated *cis*-conformation of the *N*-methylamide by the pyrrole moiety. FR193144 **49** exhibits excellent binding affinity for human recombinant B_2 receptors (IC_{50}=0.26 nM), thereby proving the *cis*-conformation as the bioactive conformation of the *N*-methylamide bearing antagonists (Fig. (**18**)) [138].

Interestingly, only minor variations within the core structure of the B_2 antagonists resulted in an analogue, FR190997 **50** (Fig. (**18**)), exhibiting an agonistic profile [143]. The agonistic behaviour is hypothesized to be encoded in the difference concerning the 4-substituent of the quinoline moiety within the agonist compared to the antagonists (H ⇨ 2-pyridylmethoxy). FR190997 **50** induces hypotensive response in anaesthetized rats and thus, is claimed for the treatment of hypertension, renal failure, heart failure, circulatory disorders, angina, restenosis, hepatitis etc [143].

B_2 Antagonists Structurally Related to FR173657

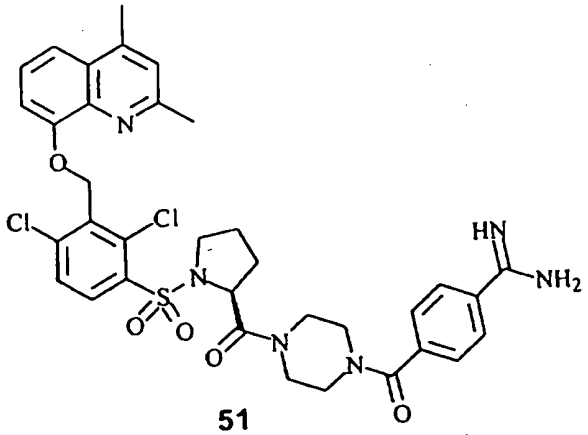
Compounds evaluated at Fournier are structurally related to Fujisawa's quinoline series differing mainly in the substituent in 3-position of the benzene-linkage which is replaced by a sulfonamide. LF16-0335 **51** (Fig. (**19**)) is a potent, selective and competitive antagonist of the human B_2 receptor, displacing [^{3}H]BK binding to membrane preparations of CHO cells expressing cloned human B_2 receptors with a K_i value of 0.84 nM.

LF16-0335 **51** shows neither affinity for the B_1 receptor, nor binds significantly to any other membrane receptor except the muscarinic M2 (IC_{50}=0.9 μM) and M1 (IC_{50}=1.0 μM) receptors [144]. The hydrochloride of this derivative, LF16-0335C, inhibits competitively BK-induced contractions of isolated rat uterus and GPI in functional assays [145]. Given *i.v.*, LF16-0335C inhibits BK-induced hypotension in both animal species in a dose-dependent manner [145]. Substitution of the piperazine ring in LF16-0335 **51** against a diaminopropane unit led to LF16-0687 **52** (Fig. (**19**)) which was shown in competition binding studies with [^{3}H]BK to bind to the human recombinant B_2 receptor expressed on CHO cells with an K_i value of 0.67 nM (LF16-0335 **51**, K_i=0.84 nM). It functions as a competitive antagonist of BK-mediated contractions in isolated organs, i.e. rat uterus and GPI. Contrary to LF16-0335 **51**, LF16-0687 **52** showed selectivity for the B_2 receptor in binding and functional studies performed on more than 40 different receptors.

In a new series of patent applications, Hoechst claimed a number of derivatives based on the lead structures delineated by Fujisawa as potent B_2 receptor antagonists. These heteroarylbenzyl ethers belong to a series of *O*-substituted 8-quinolines or 4-benzothiazoles [146]. Heitsch *et al.* report that the potency of the quinoline series was found to be higher compared to the corresponding benzothiazoles. The most potent antagonist **53** (Fig. (**20**)) shows an IC_{50} value of 0.7 nM for the inhibition of specific binding of [^{3}H]BK to GPI membrane preparations and an EC_{50} value of 4.1 nM for the inhibition of BK-induced contraction of isolated GPI.

The most potent corresponding antagonist of the benzothiazole series **54** (Fig. (**20**)) exhibits an IC_{50} value of 10.3 nM and an EC_{50} value of 54 nM. Another representative example of the B_2 antagonist claimed by Hoechst is compound **55** (Fig. (**20**)) which incorporates a 2-aminoethanol unit instead of the *N*-methylamide as linker in the central part of the molecule. **55** inhibits [^{3}H]BK binding (GPI) with a K_i value of 20 nM [127,128].

Based on the template FR173657 **48**, Kyowa Hakko filed a patent application claiming heteroarylbenzyl ethers as B_2 antagonists [147]. Like in FR173657 **48**, the central ether entity is flanked by a terminal quinoline and a dichlorobenzene linker. Instead of the classical *N*-methylamide sidechain in 3 position, the dichlorobenzene linker bears a branched hydrocarbon chain (**56**, Fig. (**20**)).



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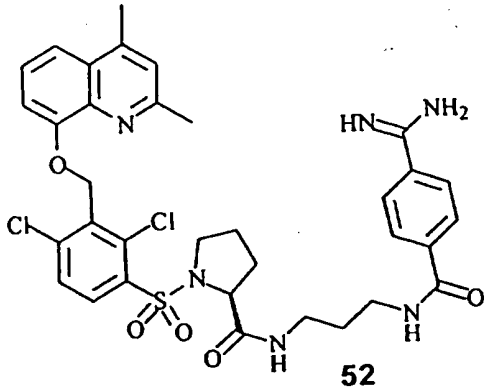

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Fig. (**19**). B_2 receptor antagonists disclosed by Fournier.

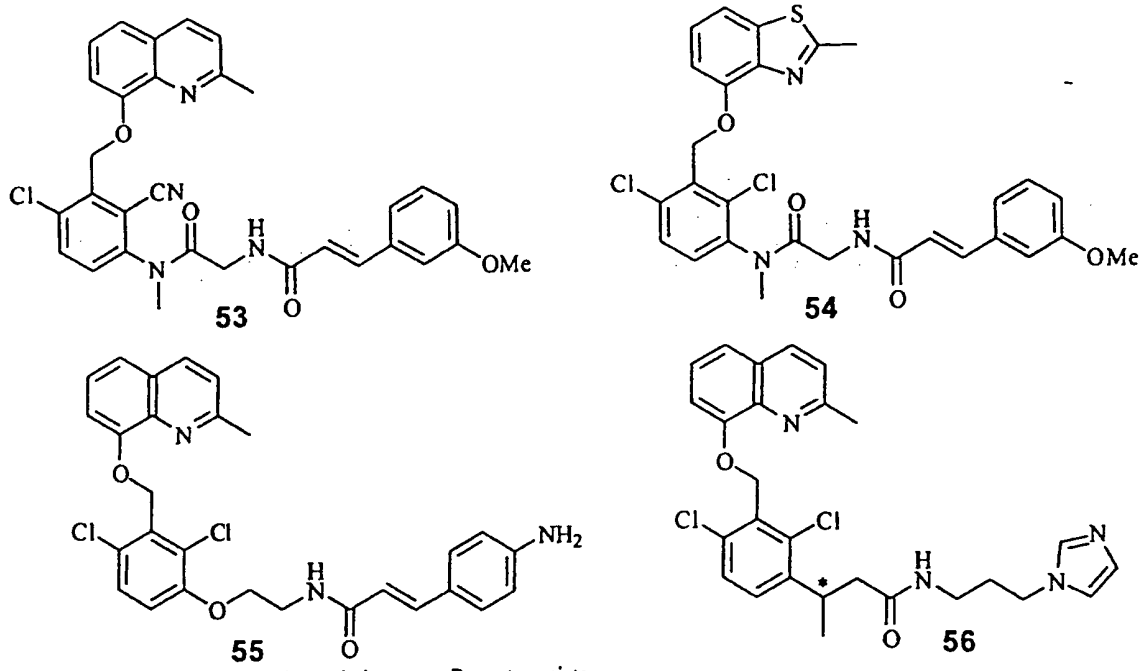


Fig. (20). Miscellaneous heteroarylbenzylether-type B_2 antagonists.

Miscellaneous Nonpeptide B_2 Antagonists

From screening of a 4000 compound combinatorial library, GlaxoWellcome found a promising tetrahydroisoquinoline, GR213548X 57 (Fig. (21)), with affinity for the B_2 receptor in the micromolar range [127].

Further B_2 antagonists are claimed in a series of patent applications by a number of companies. American Home Products (AHP, Wyeth Ayerst) presented compound 58 which structurally resembles the Fujisawa derivatives only with respect to a quinoline entity. Pfizer described 1,4-dihydropyridines such as 59 to act as B_2 antagonists, while Eli Lilly disclosed benzothiophenes 60 (Fig. (21)) [127,148-150].

Neurokinin

Biomedical Significance

Neurokinins (NKs), also termed tachykinins belong to a family of peptides sharing a common homologous *C*-terminal fragment composed of the pentapeptide amide Phe-

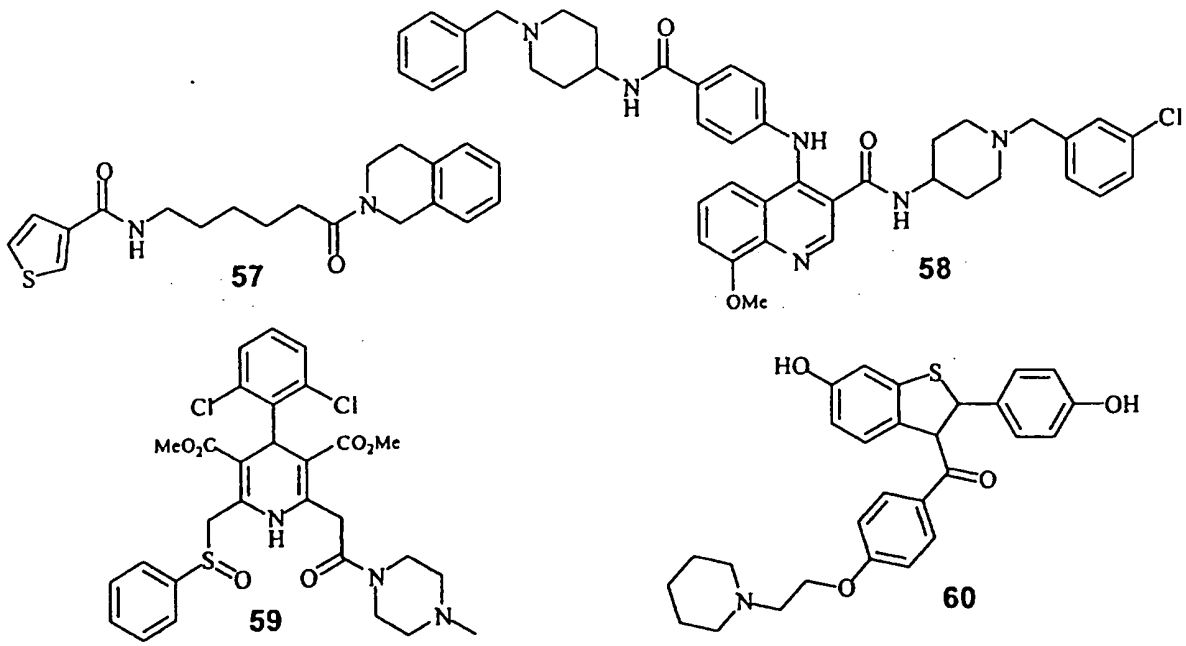


Fig. (21). Miscellaneous B_2 antagonists.

Xaa-Gly-Leu-Met-NH_2 (Table 1) [151]. The interaction of substance P (SP), neurokinin A (NKA) , and neurokinin B (NKB) with their corresponding receptors [152], notably NK_1, NK_2, and NK_3 plays a pivotal role in induction and progression of inflammatory diseases. Neurokinin interaction is involved in a variety of physiological and pathophysiological conditions such as pain, inflammation, smooth muscle contraction, vasodilation, and activation of the immune system. Thus, NK receptor antagonists emerged as interesting agents for the treatment of primarily pain, emesis and asthma but also to interfere in other disorders such as anxiety, arthritis, migraine, cancer and schizophrenia [153-156]. NK receptor antagonists have been reviewed e.g. by Elliot and Seward [157], von Sprecher *et al.* [158], and, only recently, in *Current Medicinal Chemistry* by Gao and Peet [159]. Therefore, this contribution will solely focus on nonpeptide NK antagonists.

Lead Finding

NK_1 Antagonists

The quinuclidine-based analogue CP-96,345 **61** (Fig. (**22**)) was developed from a lead structure found by random screening and is the first nonpeptide NK_1-selective antagonist showing an IC_{50} value of 0.77 nM (lymphoblast IM-9 cells) [160]. Over the last years, CP-96,345 **61** evolved as the main pharmacological tool in the area of NK receptor research.

A second series of piperidine-containing analogues developed at Pfizer includes CP-99,994 **62** [161] and CP-122,721 **63** (Fig. (**22**)) [162]. CP-99,994 **62** exhibits analgesic efficacy [163] and shows less *in vivo* inhibition of NK_1 receptor-mediated responses compared to the 5-trifluoromethoxy analogue, CP-122,721 **63** [164]. The latter

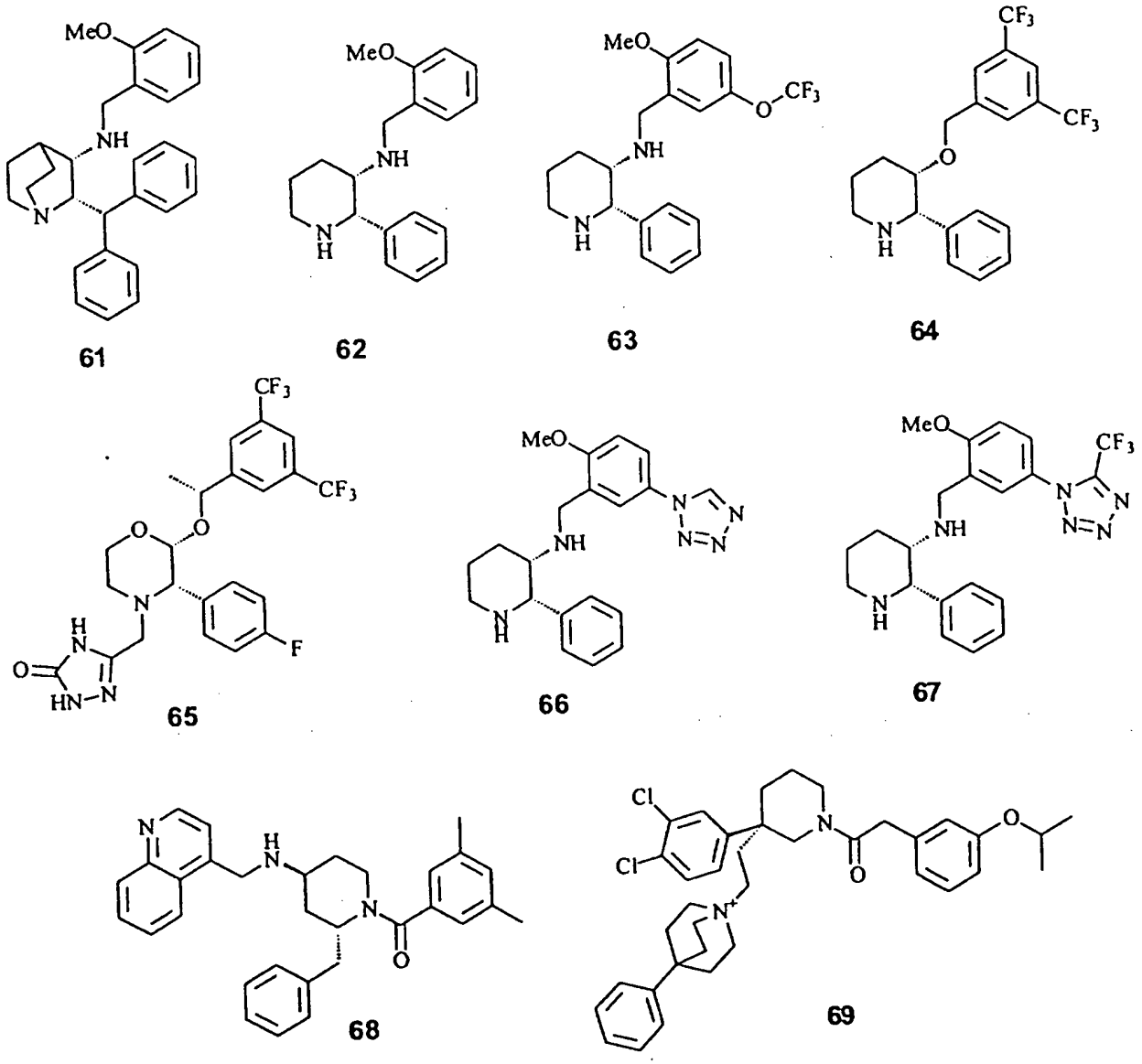
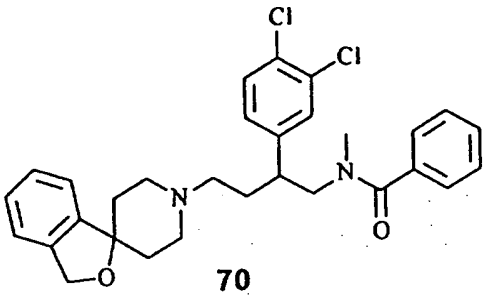


Fig. (22). Quinuclidine-, piperidine-, and morpholine-derived NK_1 antagonists.



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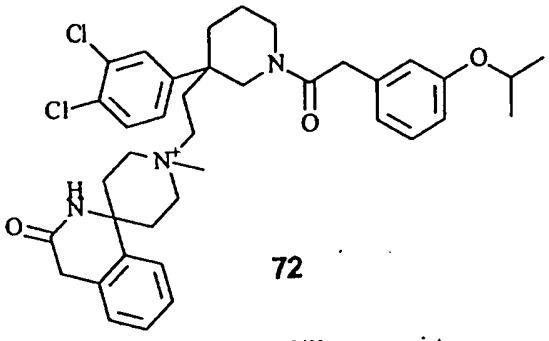

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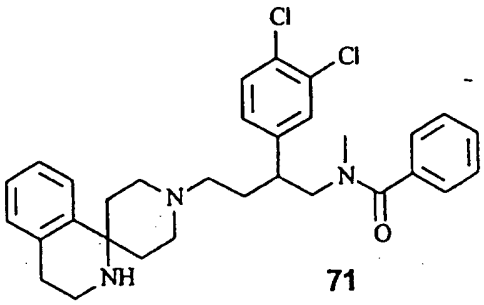

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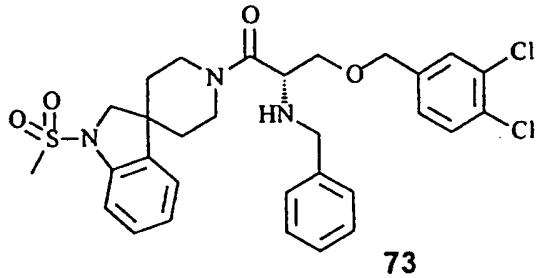

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Fig. (23). Spiro-aryl piperidine-type NK_1 antagonists.

congener shows improved antiemetic properties in acute cisplatin-induced vomiting in tumor patients when administered in combination with a 5-HT_3 antagonist [157].

Based on the piperidine core structure of CP-99,994 **62**, Merck synthesized L-733,060 **64** (IC_{50}=0.87 nM in CHO cells) [165] which, after modifications, led to the metabolically more stable L-754,030 **65** (IC_{50}=0.1 nM in CHO cells) (Fig. (22)) [166]. Recent results indicate that L-754,030 **65** prevents cisplatin-induced emesis in patients receiving an anticancer chemotherapy [167,168].

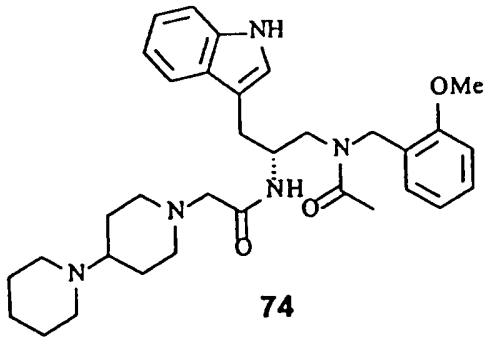
Glaxo disclosed the 5-tetrazolyl-substituted analogue GR-203,040 **66** (Fig. (22)) retaining the piperidine core structure of CP-99,994 **62** as NK_1 antagonist (GR-203,040 **66**: pK_i=10.3 nM in CHO cells) which was selected for clinical evaluation in emesis and migraine [169,170]. Further modification revealed GR-205,171 **67** (Fig. (22)) (pK_i=10.6 nM in CHO cells) which, apart from oral bioavailability, exhibits also reduced L-type calcium channel activity, a side effect associated with e.g. CP-122,721 **63**. GR-203,040 **66** ameliorates tissue damage induced by x-irradiation or cisplatin [171,172].

Novartis developed CPG-49,823 **68** (Fig. (22)), based on the piperidine scaffold for anxiety-related indications [173]. CPG-49,823 **68** (IC_{50}=12 nM, bovine retina) has been tested for its antagonistic potential against the depolarization of spinal motoneurones by bath application of the selective tachykinin receptor against septide(6-11) exhibiting an IC_{50} value of 0.3 μM (gerbil preparations) and 7.8 μM (rat preparations) [174].

The central piperidine unit is also found in the Sanofi compound SR-140,333 **69** (Fig. (22)) (IC_{50}=0.01 nM in IM-9 cells), also termed Nolpitantium, which emerged from a random screening approach followed by a lead optimization program [175].

Investigations on the effects of SR-140,333 **69** on nociceptive pathways in rats revealed this agent to be a potent drug for pain relief [176]. Kubota *et al.* reported on the synthesis of spiro-piperidines as NK_1 receptor antagonists [177]. SAR studies starting from the primary lead YM-35375 **70** (dual NK_1/NK_2 antagonist) (Fig. (23)) yielded analogue YM-35384 **71** as a selective NK_1 antagonist which was 12-fold more potent compared to the original spiro-isobenzofuran-1(3*H*)-4'-piperidine YM-35375 **70**. YM-35384 **71** already showed an IC_{50} value of 58 nM which could be improved by further modification resulting in compound YM-49244 **72** (Fig. (23)), a spiro-substituted piperidinium salt with an IC_{50} value of 1.9 nM against SP-induced contraction in guinea pig ileum and inhibitory activity against selective NK_1 receptor agonist-induced bronchoconstriction in guinea pigs (ID_{50}=24 μg/kg, *i.v.*) [177].

A further class of spiro-aryl piperidines is represented by Merck Sharp and Dohme's spirocyclic aryl sulfonamides, serine-derived NK_1 antagonists [178]. Compound **73** (Fig. (23)) exhibits an IC_{50} value of 1.0 nM for the displacement of [^{125}I]SP from NK_1 receptors in CHO cells and served for

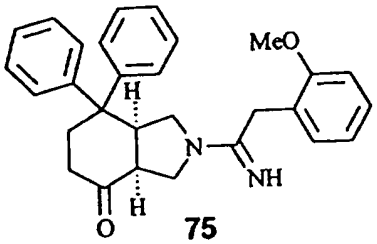


74

Fig. (24). Lanepitant disclosed by Eli Lilly.

the development of a pharmacophore model for the receptor binding requirements [179].

Eli Lilly has identified the tryptophane-derived LY-303,870 74 (Fig. (24)) as a selective antagonist binding to NK_1 with high affinity, while lacking ion channel activity [180]. LY-303,870, Lanepitant 74, is a candidate for clinical development in animal models of inflammation, pain, migraine, and asthma [158].



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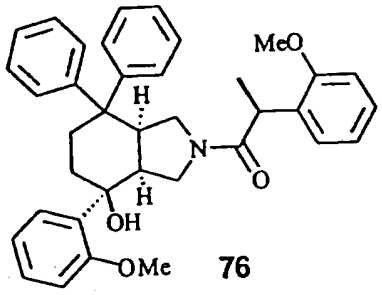

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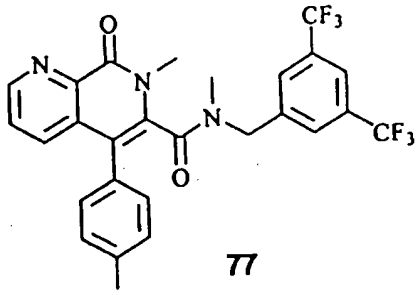
Fig. (25). Perhydroisoindole-type NK_1 antagonists.

RP-67,580 75 (Fig. (25)) emerged after lead optimization of an initial screening hit of Rhône-Poulenc Rorer's compound stock. RP-65,580 75 belongs to a class of substituted perhydroisoindoles which, apart from poor oral bioavailability, also suffered from L-type calcium channel interaction [151,181]. The follow-up compound RPR-100,893 76 (Fig. (25)), Dapitant, exhibits superior binding affinity (IC_{50}=13 nM, IM-9 cells) [182].



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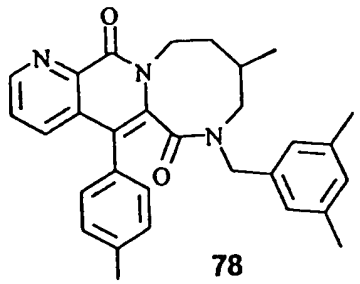

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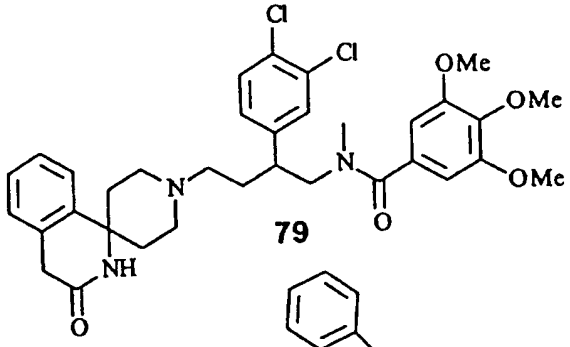
Fig. (26). Naphthydrine-type NK_1 antagonists.

Investigations of the axially chiral 1,7-naphthydrine-6-carboxamide 77 (Fig. (26)) revealed that the atropisomer (*aR*)-trans-77 represents the bioactive receptor-bound conformation of this potent NK_1 antagonist [183]. This analogue exhibits *in vitro* antagonistic activities for the inhibition of [^{125}I]Bolton-Hunter(BH)-SP binding in human lymphoblast cells (IM-9) with an IC_{50} value of 0.24 nM. Further, it shows *in vivo* potency by inhibiting capsaicin-induced plasma extravasation in the trachea of guinea pigs upon *i.v.* and *p.o.* administration.



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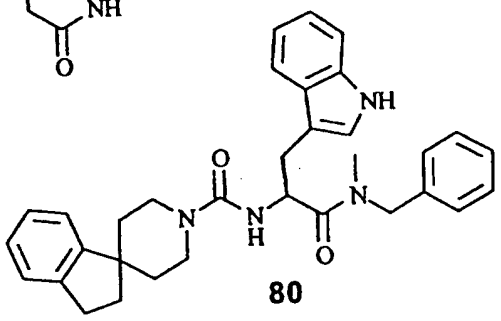

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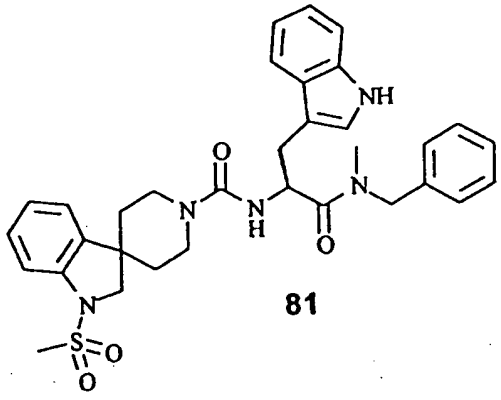

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Fig. (27). Dual NK_1/NK_2 antagonists.

Based on this template, Natsugari *et al.* [183] developed TAK-637 78 (Fig. (26)), the (*aR*,9*R*)- atropisomer of a cyclic naphthyridine analogue. TAK-637 78 exhibits an IC_{50} value of 0.45 nM, an ID_{50} of 4.3 μg/kg and 33 μg/kg after *i.v.* and *p.o.* administration, respectively. Further it increased the shutdown time of distension-induced bladder contractions and the bladder volume threshold in guinea pigs, thus implying its clinical potential in the treatment of pollakiuria and urinary incontinence [183]. The x-ray structures of 77 and 78 provide insights in the prerequisite structural

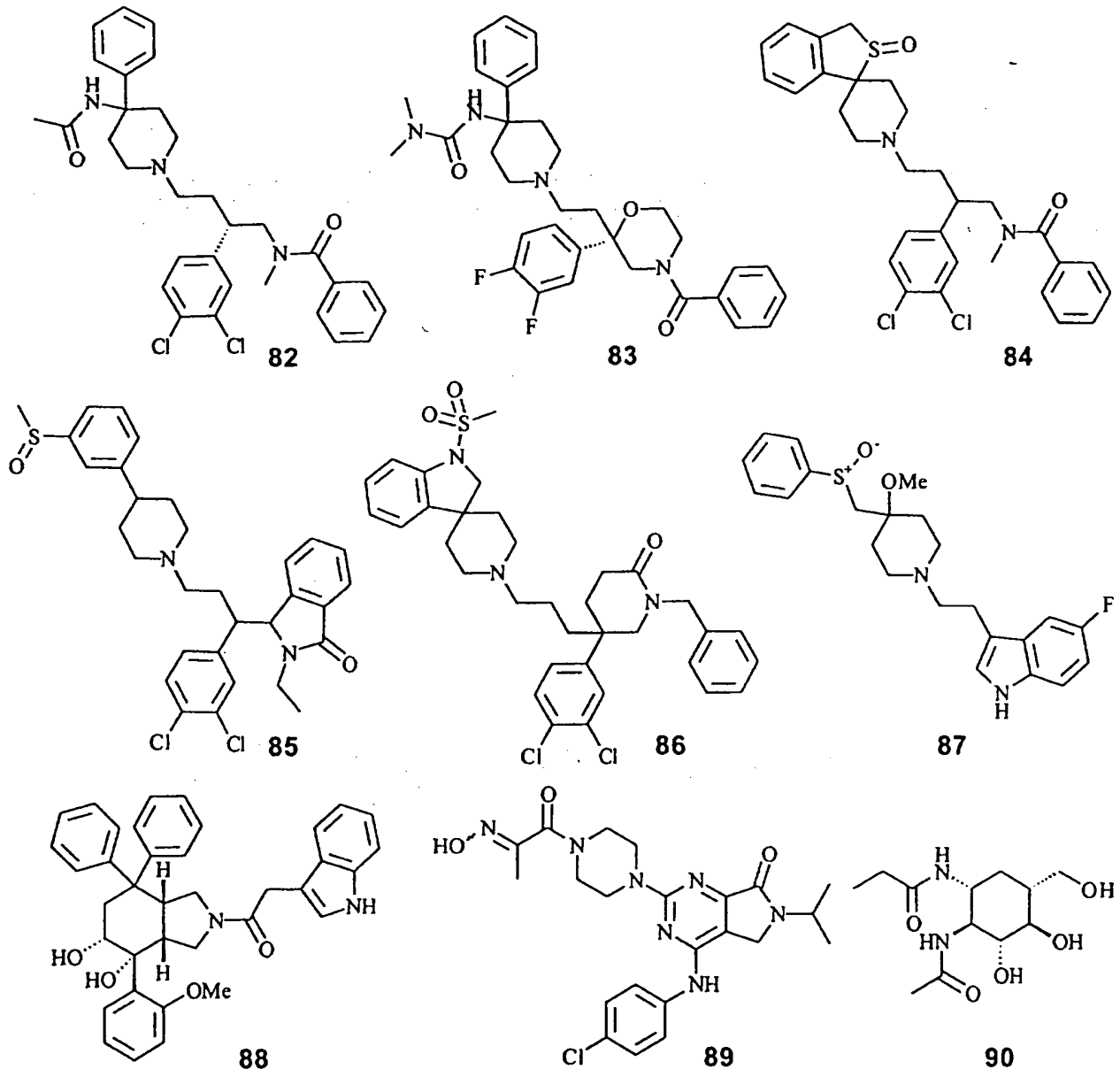


Fig. (28). NK_2 antagonists.

requirements for NK_1 receptor binding, thereby assigning the (*aR*,9*R*)-isomer as the active conformation [183].

Dual NK_1/NK_2 Antagonists

Since the release of SP and NKA causes mucus secretion, airway constriction, and plasma extravasation - typical clinical symptoms of asthma - it has been suggested to use dual NK_1/NK_2 antagonists in the treatment of asthma [184].

Considering the structural requirements of Sanofi's NK_2-selective antagonist SR-48968 **82** (Fig. (28), see below), researchers at Yamanouchi Pharm. developed the spiro[isobenzofuran]piperidine YM-35375 **70** (Fig. (23)) with binding affinity towards the NK_2 receptor with an IC_{50} value of 84 nM and an IC_{50} value 710 nM for NK_1, respectively. Further, it shows inhibitory activity (ID_{50}=41 μg/kg, *i.v.*) against [β-Ala^8]NKA(4-10)-induced bronchoconstriction in guinea pigs [185]. Utilizing this new NK_1/NK_2 dual antagonist as lead compound a further spiro-substituted piperidine analogue, YM-44778 **79** (Fig. (27)), was developed, exhibiting potent antagonistic activities against the NK_1 (IC_{50}=82 nM) and NK_2 (IC_{50}=62 nM) receptors in isolated tissues [185], respectively.

Based on L-tryptophanebenzyl esters, Qi *et al.* reported on the synthesis of two compounds **80**, and **81** with dual NK_1/NK_2 receptor affinity (Fig. (27)) [186].

80 contains a 4-spiroindano piperidine and shows dual NK activity combined with slightly improved NK_2 activity (IC_{50}=56 nM (hNK_1), IC_{50}=27 nM (hNK_2)). Upon incorporation of a 4-spiroindolin sulfonamide, the balanced antagonist **81** was obtained (IC_{50} = 14 nM - NK_1; 24 nM - NK_2).

NK_2 Antagonists

NK_2 antagonists are of particular interest for the treatment of chronic diseases such as asthma, inflammatory bowel disorders, rheumatoid arthritis, pain, emesis, and psychiatric disorders [157].

The first NK_2 antagonist, SR-48,968 **82** (Fig. (**28**)), Saredutant, was described in 1992 [187]. This potent antagonist has been shown to inhibit the NKA-induced brochoconstriction in isolated human airways. Only recently, a study of van Schoor *et al.* have demonstrated that NKA-induced bronchoconstriction in asthmatics was significantly reduced with 100 mg Saredutant administered *p.o* [188].

Based on this prototype compound, a number of analogues emerged from different laboratories. SR-144,190 **83** (Fig. (**28**)) retains the phenylpiperidine moiety but contains an additional morpholine unit in order to introduce rigidity. Compared to the parent compound, it exhibits a similar pharmacological profile with increased bioavailability in the CNS [189].

Also Yamanouchi (YM-38336) **84** and Zeneca (ZD-7944) **85** (Fig. (**28**)) presented potent NK_2 antagonists based on the Sanofi lead structure (SR-48,968 **82**). ZD-7944 **85** [190], showing a K_i value of 0.14 nM (MEL cells), still retains the phenylpiperidine entity, while YM-38336 **84** [191] has been modified by introduction of a spiro-benzothiophene residue in position 4 of the piperidine. YM-38336 **84** shows potent NK_2 inhibitory activity against [β-Ala^8]NKA(4-10)-induced bronchoconstriction in guinea pigs, demonstrated by an ID_{50} value of 20 mg/kg, *i.v.* [192].

Harrison *et al.* reported on the development of selective NK_2 and NK_3 antagonists based on a common structural template, notably the NK_3-selective compound SR-142,801 **91** (Fig. (**29**), see below) [193]. Transfer of the carbonyl oxygen from an exocyclic to an endocyclic position on the piperidine ring led to two series of selective analogues, NK_2 and NK_3 antagonists, respectively [193]. An example of a potent NK_2 antagonist is given by compound **86** (Fig. (**28**)) which exhibits an IC_{50} value of 2.2 nM for the displacement of [^{125}I]NKA from the cloned human NK_2 receptor in CHO cells.

A number of preclinical nonpeptide NK_2 antagonists have been reported by GlaxoWellcome, Rhône-Poulenc Rorer and Zeneca, e.g. GR-159,897 **87**, RPR-106,145 **88** (related to the NK_1 antagonist RPR-100,893 **76**, (Fig. (**25**))), and ZM-253,270 **89** (Fig. (**28**)) [158], respectively.

Menarini used an interestingly rigid template for its selective NK_2 antagonists (K_i=2.5 nM) MEN-11420 **90**, Nepadutant, exhibiting improved *in vivo* potency and duration which is attributed to its rigid structure [194].

NK_3 Antagonists

The first selective nonpeptide NK_3 antagonist, SR-142,801 **91**, Osanetant, has been reported by Sanofi (K_i=0.21 nM, CHO cells) (Fig. (**29**)) [195].

Based on this structural template, Merck Sharp and Dohme elaborated a series of NK_2 and NK_3 antagonists, exemplified with analogue **92** (Fig. (**29**)), the corresponding congener of **86** (Fig. (**28**)).

SmithKline Beecham claimed NK_3 antagonists for the treatment of CNS diseases, pulmonary disorders and dermatitis [196]. Based on a quinoline core structure,

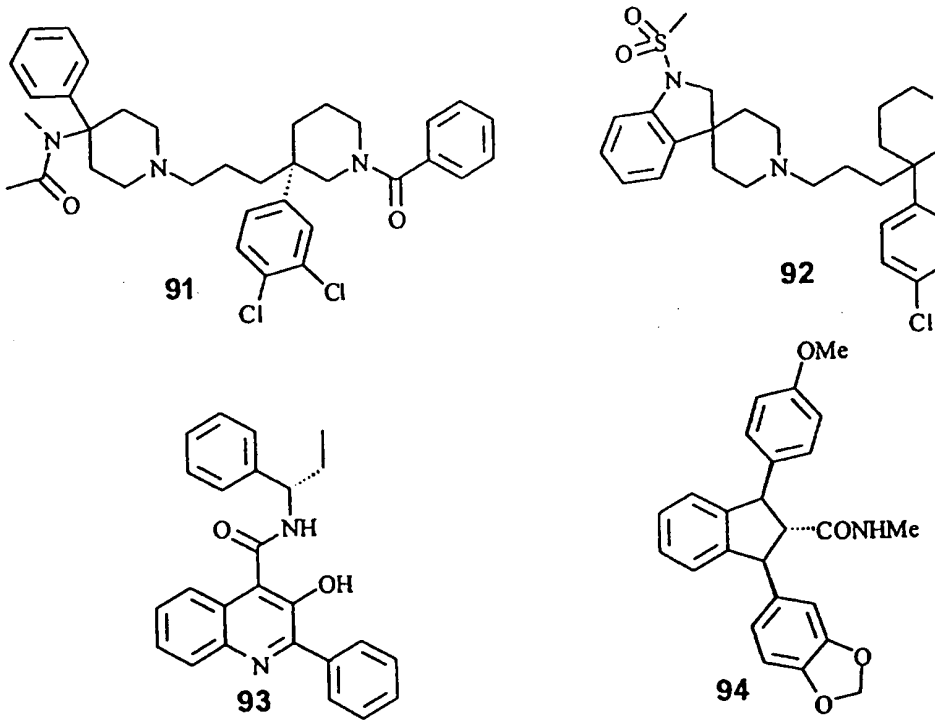


Fig. (**29**). NK_3 antagonists.

Giardina *et al.* developed SB-223,412 **93** (Fig. (29)) demonstrating high NK_3 activity (IC_{50}=1.2 nM, K_i=1.0 nM, CHO cells), weak NK_2 activity, and no affinity for other receptors including ion channels [197]. SB-223,412 **93** exhibits *in vitro* and *in vivo* oral and intravenous activity in animal models [198].

An entirely novel structure, **94** (Fig. (29)), has been claimed as NK_3 antagonist for the treatment of bronchitis, asthma, anxiety, Parkinson's disease and dermatitis [199]. Interestingly, this compound resembles strongly the indane carboxylic acids of SmithKline Beecham's ET antagonists.

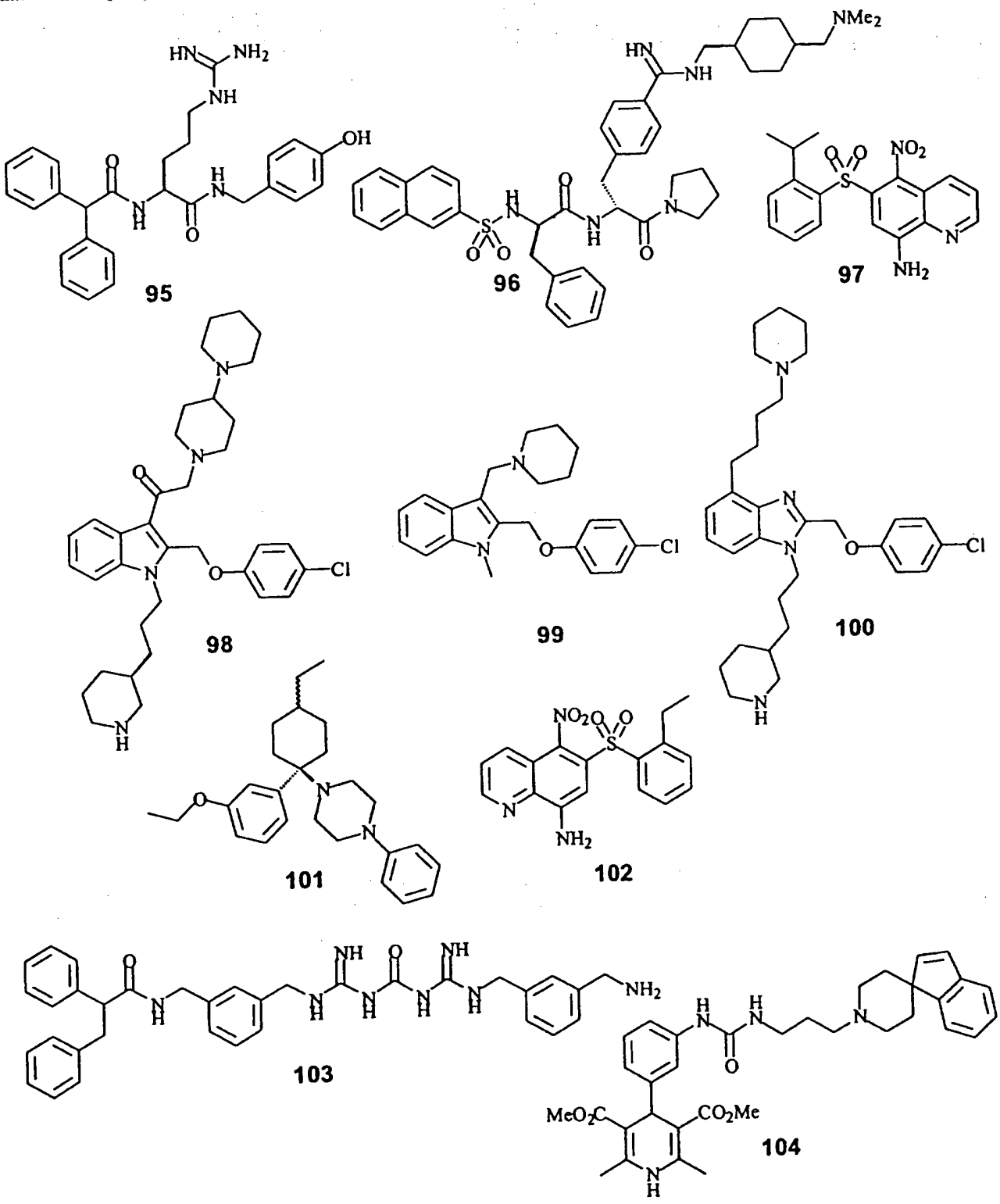


Fig. (30). Miscellaneous Y_1 antagonists.

Neuropeptide Y

Biomedical Significance

The 36-amino acid peptide neuropeptide Y (NPY, Table 1) was discovered in 1982 by Tatemoto *et al.* [200]. NPY is a member of the pancreatic polypeptide family, also including structurally related peptide YY (PYY) and pancreatic peptide (PP) [201]. NPY is widely distributed throughout the mammalian central and peripheral nervous system [202,203]. Interacting with its at least six receptor subtypes (Y_1-Y_6) it is involved in numerous physiological functions, e.g. food intake, blood pressure regulation, hormone secretion, sexual behaviour, and circadian rhythm [204-209]. Patent literature issued over the last ten years concentrate mainly on the inhibition of receptor-ligand interactions by low-molecular weight compounds in order to therapeutically interfere in mechanisms such as anxiety, appetite stimulation, obesity, alcohol intake, hypertension, and regulation of coronary tone [210]. As the Y_1 and Y_5 receptors are suggested to control feeding behaviour, they are believed to be the best target systems for developing antagonists as therapeutics for the treatment of obesity [204,211-213]. The Y_1 receptor, found in the peripheral and in the central nervous system (CNS), has been cloned in 1992 [214]. Its modulation may influence numerous physiological conditions including anxiety, diabetes, obesity, or appetite disorders. Most recently, the Y_5 receptor has been cloned and characterized to be involved in food intake regulation [212]. A review published by Ling in 1999 reports on the patent situation related to NPY antagonists [210]. In this contribution representative examples of potentially active nonpeptide NPY antagonists will be described according to their target receptors.

Y_1 Receptor Antagonists

A number of Y_1 antagonists (Fig. (**30**)) published over the last ten years show binding affinities in the nanomolar range, e.g. as BIBP3226 **95** (K_i=7.2 nM), SR120819 **96** (K_i=15 nM), PD160170 **97** (K_i=48 nM), and LY-357897 **98** (K_i=0.75 nM) (Fig. (**30**)) [215-218]. The best characterized Y_1 antagonist BIBP3226 **95** has been demonstrated to inhibit NPY-mediated vasoconstriction and pressure variations [215]. SR120819 **96** represents a dipeptide analogue containing a sulfonamide. This orally active antagonist incorporating a central arginine mimic (benzamidine in **96**) develops its potency in the 1,4-*cis*-disubstituted cyclohexyl ring by antagonizing NPY-mediated pressure responses [219].

Parke-Davis discovered a new and unique class of moderately potent but selective Y_1 antagonists by random screening of which PD160170 **97** is a representative compound. Eli Lilly described LY-357897 **98** from a series of trisubstituted indoles and benzimidazoles. Compound **99** (Fig. (**30**)) [220] showing a K_i value of 2.1 μM was discovered by a biased screening of the in-house library and served as lead structure in the subsequent SAR studies of the trisubstituted indole series. Consequent structure modification led to **98**, the most active analogue (K_i=0.75 nM), which, in (*S*)-configuration inhibits NPY-induced forskolin-stimulated cAMP release and intracellular Ca^{2+} release in the nanomolar range. The corresponding

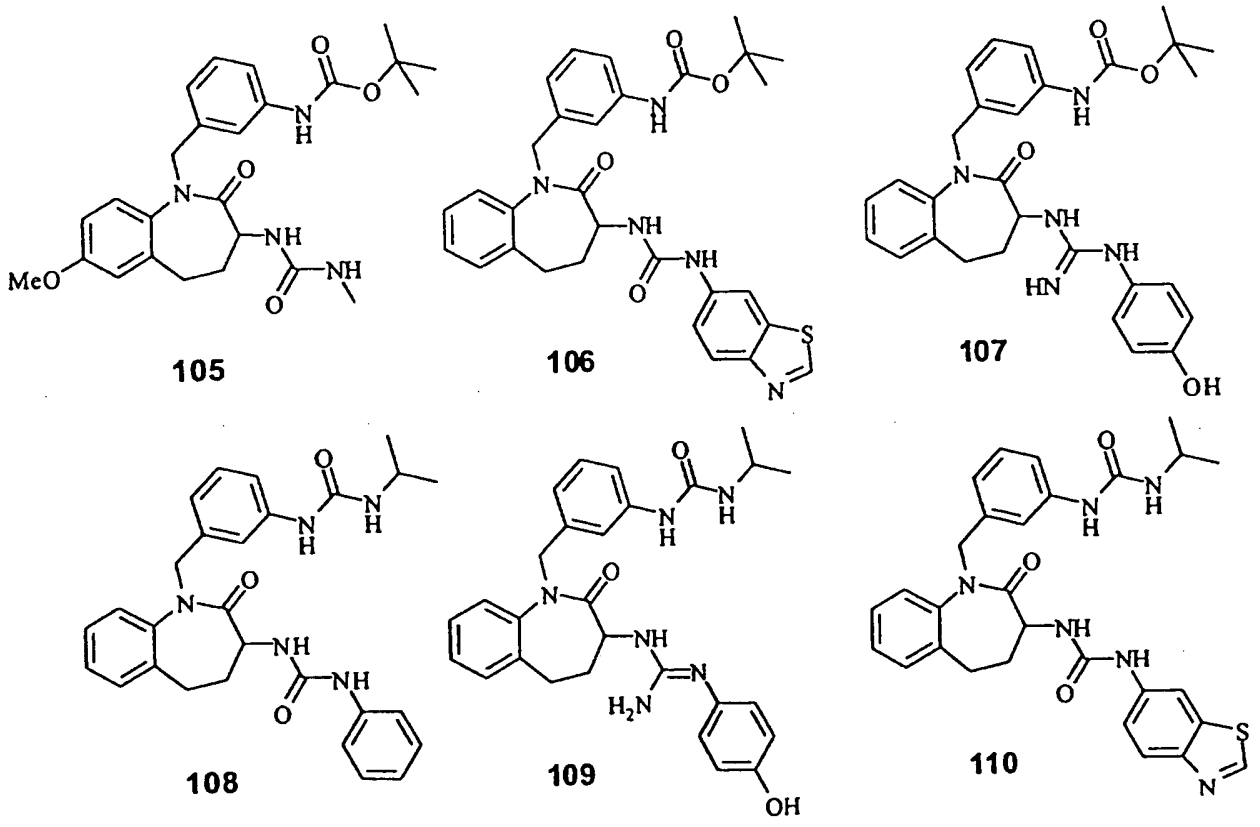


Fig. (31). Benzazepinone-type Y_1 antagonists.

benzimidazole series has also been investigated [221]. A representative example is given by compound **100** (Fig. (**30**)) which was obtained after systematic optimization of the N1- and C4-substituents of the benzimidazole scaffold. Compound **100** exhibits *in vitro* binding affinity on AV-12 cells expressing the human Y_1 receptor with a K_i value of 1.7 nM.

Pfizer claimed a series of piperazinyl-comprising compounds as Y_1-selective antagonists [222]. Analogue **101** (Fig. (**30**)) demonstrates an interesting activity profile by expressing a differentiated behaviour of the two conformers, i.e. *cis*- (IC_{50}=76 nM) and *trans*- (IC_{50}=525 nM) exposed ethyl substituent with respect to the phenylpiperazine substitutent of the cyclohexyl ring.

Warner Lambert filed compounds based on a quinoline scaffold that were claimed as Y_1 subtype selective antagonists. The 6-aryl-sulfonyl-quinoline analogue **102** (Fig. (**30**)) inhibits [^{125}I]PYY binding to the human Y_1 receptor with an K_i value of 48 nM [223].

Alanex Corp. claimed two series of compouds containing either an amidino-urea or a diamidino-urea core structure. A representative of the latter series is given by **103** (Fig. (**30**)) inhibiting the binding of [^{125}I]PYY to the Y_1 receptor in membranes derived from human neuroblastoma cell lines (SK-N-MC) with an IC_{50} value of 70 nM [224].

Bristol Myers Squibb's patents enclose two structurally related compound classes, i.e. phenyl-dihydropyridines [225] and phenyl-dihydropyrimidines [226]. In compound **104** (Fig. (**30**)) the *m*-substituted phenyl-dihydropyridine sidechain is terminated with a spiroindane, a structural element which is also found among other antagonists directed against numerous members of the peptide-binding GPCR superfamily.

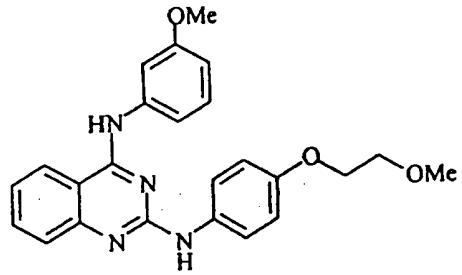
Murakami *et al.* [227] at Shionogi published a novel class of 1,3-disubstitued benzazepinones as potent and selective Y_1 antagonists. Based on the lead compound **105** (Fig. (**31**)) (K_i=1.5 μM) which emerged from a random screening approach, follow-up compounds **106** (K_i=160 nM) and **107** (K_i=39 nM) have been obtained (Fig. (**31**)).

Further optimization of the phenyl substituent in position 3 leading to analogue **108** as well as optimization of the substituent in position 3 of the 2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one, represented by congener **109** (Fig. (**31**)) resulted in an increase of the binding affinity towards 43 nM and 2.9 nM, respectively. Combination of the optimized structural features led to one of the most potent derivatives (**110**, Fig. (**31**)) which competitively inhibits specific [^{125}I]PYY binding to Y_1 receptors in human SK-N-MC cells with a K_i value of 5.1 nM. Although **110** also antagonizes the Y_1 receptor-mediated increase in cytosolic free Ca^{2+} concentration in SK-N-MC cells, it has not been evaluated *in vivo* because of its poor solubility in aqueous solution and poor oral bioavailability. Hence, it has been shown in binding assays with 17 receptors including the Y_2, Y_4, and Y_5 receptor that it binds selectively to the Y_1 receptor [227].

Y_5 Receptor Antagonist

Several patent applications have been filed by Novartis in 1997 [228-230] claiming diamino quinazolines as selective Y_5 antagonists. They were shown to inhibit NPY-induced Ca^{2+} increase in stable transfected cells expressing the Y_5 receptor. Analogue **111** (Fig. (**32**)) decreases food intake by 60% in 24 h food deprived rats after *i.p.* administration of 30 mg/kg.

In 1998 Banyu Pharm. [231,232] and Bayer [233] filed patents including aminopyrazoles, aminopyridines and an amide based core structure as Y_5 antagonists. The Banyu compounds **112** and **113** showed IC_{50} values for Y_5 binding of 8.3 nM and 4.1 nM, respectively [234], whereas the Bayer compound **114** binds with an IC_{50} value of 0.47 nM. Also this congener shows selective affinity for the Y_5 receptor compared to Y_1, Y_2, or Y_4 receptor subtypes (Fig. (**32**)) [234].



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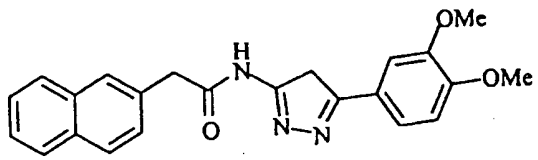

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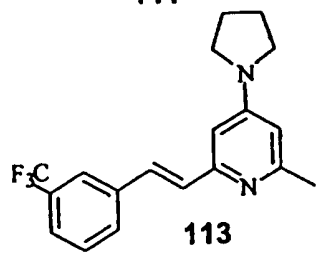

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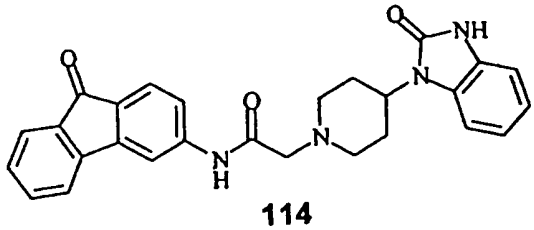


114

Fig. (**32**). Y_5 antagonists.

STRUCTURAL-BASED DRUG DESIGN

After having addressed the classical lead finding approach characterized by screening compound libraries with subsequent optimization, the complementary strategy of structure-based design will be highlighted, since this strategy is about to change the classical paradigm of "random *versus* rational" in favour of "random *goes* rational". Due to the fact that no high-resolution structure of any GPCR protein is available, all design attempts are still restricted on comparative analyses of structural features of biologically characterized low-molecular weight compounds which are interpreted in terms of steric and physicochemical complementarity to a hypothetical receptor binding site. Currently pursued GPCR research projects represent textbook examples for the fruitful combination of ligand-derived rationales that are incorporated into e.g. the design of combinatorial chemistry programs with the aim to direct resulting libraries more efficiently to the target class of interest, rather than attempting to explore systematically the infinite universe of molecular diversity. In the following, a few representative research efforts will be introduced that clearly attempt to change the mainstream of classical lead finding programs in favour of knowledge-based approaches.

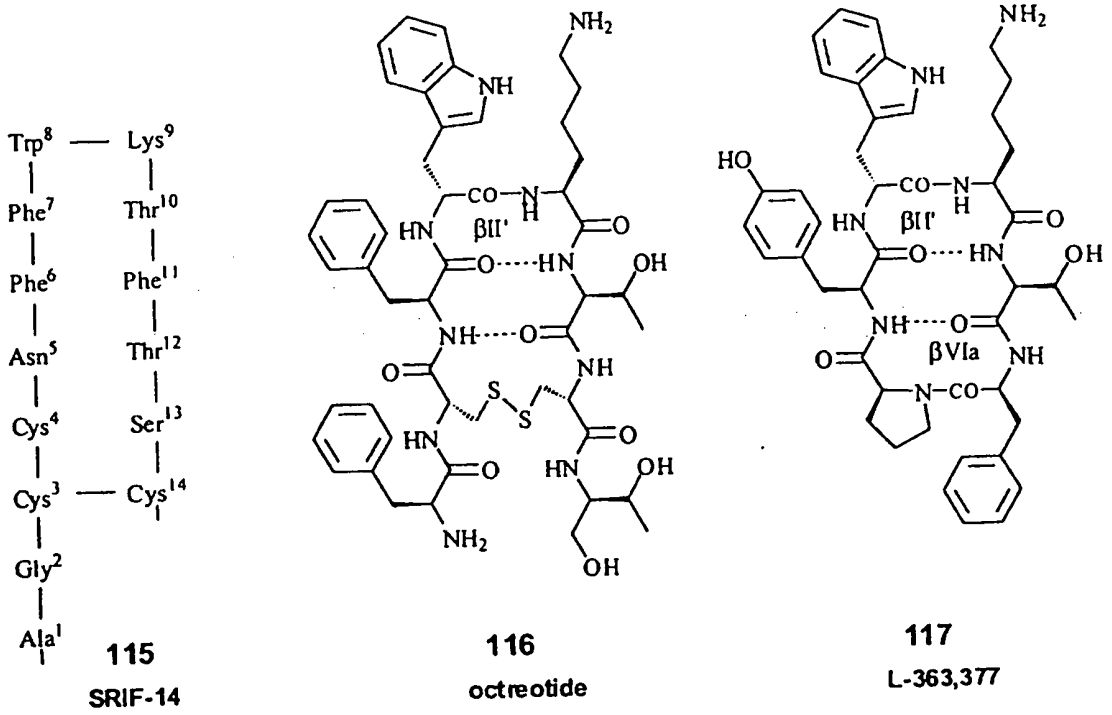
Somatostatin

Somatostatin (Somatotropin Release-Inhibiting Factor, SRIF) (Table 1) was discovered because of its inhibitory effect on growth hormone secretion. The peptide hormone which exists in two biologically active forms, the 14 amino acid form (SRIF-14) and the 28 amino acid form (SRIF-28), acts as a neuromodulator [235].

Five receptor subtypes for somatostatin (sst_1-sst_5) have been cloned and characterized from human tissue [236]. Apart from its pivotal role as neuromodulator within the central nervous system (CNS), somatostatin alters the secretion of growth hormone (GH), insulin, glucagon, pancreatic enzymes, and gastric acid [237-240]. Consequently, analogues of somatostatin emerged as interesting tools in the treatment of disorders linked to the above mentioned physiological functions. Somatostatin agonists may therefore be used for the treatment of acromegaly, diabetes, cancer, rheumatoid arthritis, and Alzheimer's disease. Especially sst_2-selective agonists emerged as useful candidates for the treatment of acromegaly, retinopathy, and diabetes [241,242].

The area of somatostatin agonist and antagonist research is a textbook example for indirect drug design utilizing ligand-derived structural rationales for design purposes. In the beginning of the 1990's numerous design projects were pursued aimed to replace the peptide scaffold of the pharmacophoric portion of somatostatin (SRIF-14) yielding a variety of moderately active, chemically diverse compounds. More recent lead finding programs employ the highly efficient technology of combinatorial chemistry for rapid modification of promising hits culminating in subtype-selective high-affinity binding compounds from a series of designed libraries. A brief overview of both, the rational design of single somatostatin-based peptidomimetics as well as the combinatorial chemistry-based approaches for lead identification and optimization will be given after a short description of the somatostatin-relevant pharmacophore hypothesis.

The tetradecapeptide SRIF-14 **115** (Fig. (33)), one of the widely distributed active forms of somatostatin, is believed



115 SRIF-14

116 octreotide

117 L-363,377

Fig. (33). Conformational preferences of somatostatin-derived peptide analogues.

to adopt a two-stranded β sheet conformation induced by a β turn encompassing Phe^7-Trp^8-Lys^9-Thr^{10}, and the disulfide bridge between Cys^3 and Cys^{14}, respectively (Fig. (33)). The conformation is further stabilized by the transannular H-bonding pattern typical for antiparallel sheet structures. From numerous sequence- and structure-activity studies it turned out that the primary pharmacophore consists of the β turn forming residues Phe^7-Trp^8-Lys^9 and an additional lipophilic binding element reminiscent to Phe^6/Phe^{11} [243].

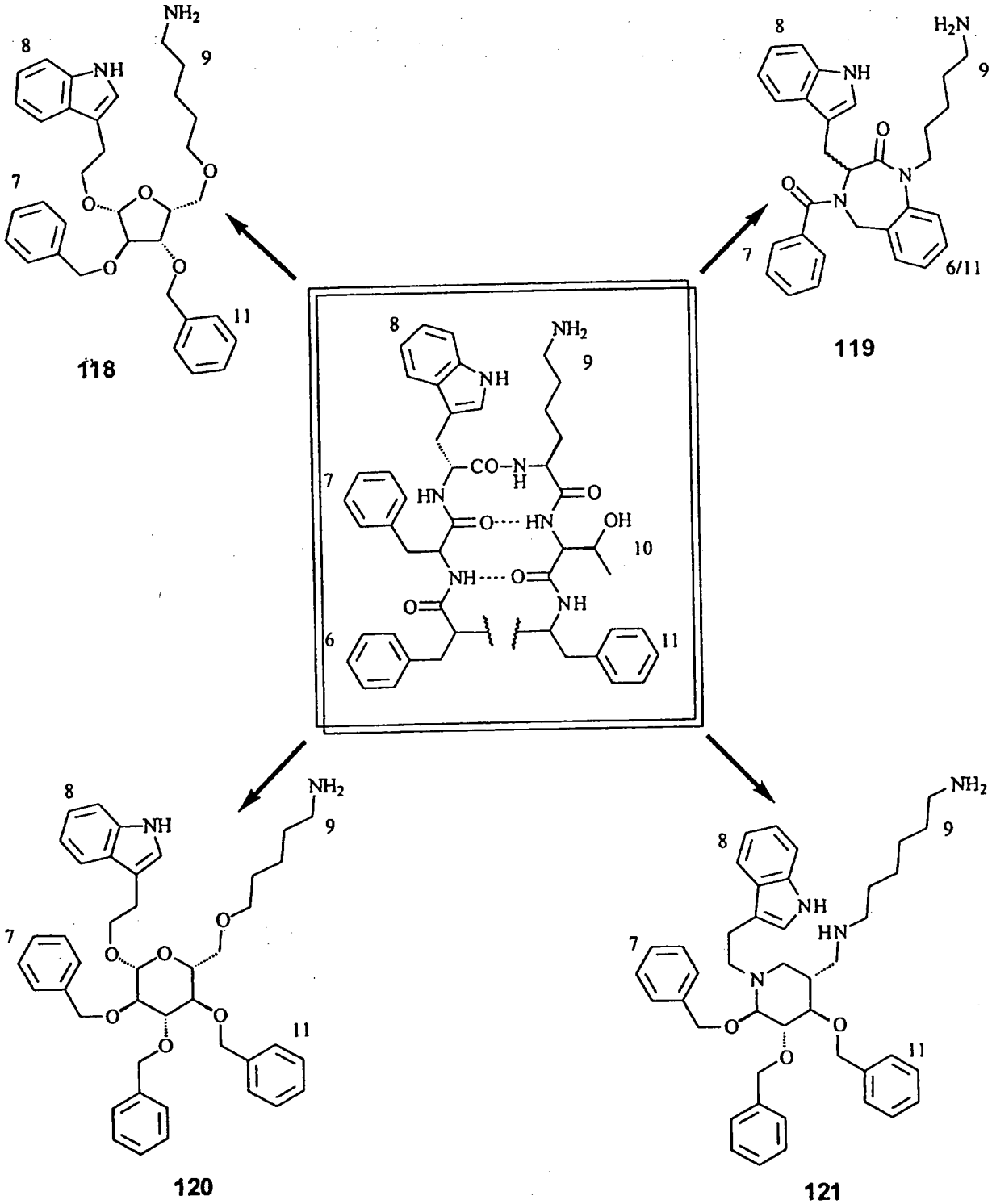


Fig. (34). Peptide conformation-derived non-peptide somatostatin antagonists. The numbering scheme refers to that of SRIF-14 (see Fig. (33)).

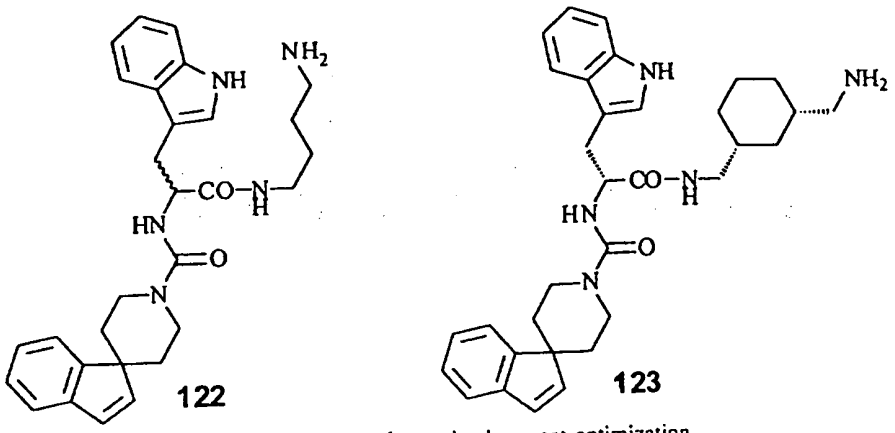


Fig. (35). High-affinity *h*sst$_2$ antagonists derived by screening and subsequent optimization.

The experimentally derived conformations of the metabolically more stable peptide analogues, e.g. octreotide (Sandostatin®) **116** [244,245] or L-363,377 **117** [246,247] not only prove the pharmacophore hypothesis, but were further used as template structures underlying a series of rational design attempts (Fig. (33)). In 1992, researchers at Sandoz designed a tetra-substituted xylofuranose derivative **118** (Fig. (34)) positioning the sidechains of Phe^7-Trp^8-Lys^9 at its C-2, C-3, and C-5 atoms, while the benzyloxy group attached to C-3 resembles the aromatic sidechain of Phe^{11}, respectively (Fig. (34)) [248].

The xylose derivative **118** displaced radio-labelled octreotide **116** from its receptor with an IC_{50} of 23 μM. Even though the mutual steric fit of the xylose-based mimic and the somatostatin structure was reasonable, the compounds displayed only moderate affinity which was attributed to the loss of considerable conformational entropy during receptor binding. Consequently, the design strategy at Sandoz was directed towards more rigid compounds based on nonpeptide scaffolds. For the purpose of substituting the peptide backbone of SRIF-14 within the β turn portion the privileged structure of the 1,4-benzodiazepinone was employed from which the pharamcophoric groups could radiate into the periphery [249]. The resulting nonpeptide tetrapeptide-mimetic **119** (Fig. (34)) was designed to account for the sidechains of Phe^7-Trp^8-Lys^9 by the appropriate substituents, while the aromatic ring of the benzodiazepine

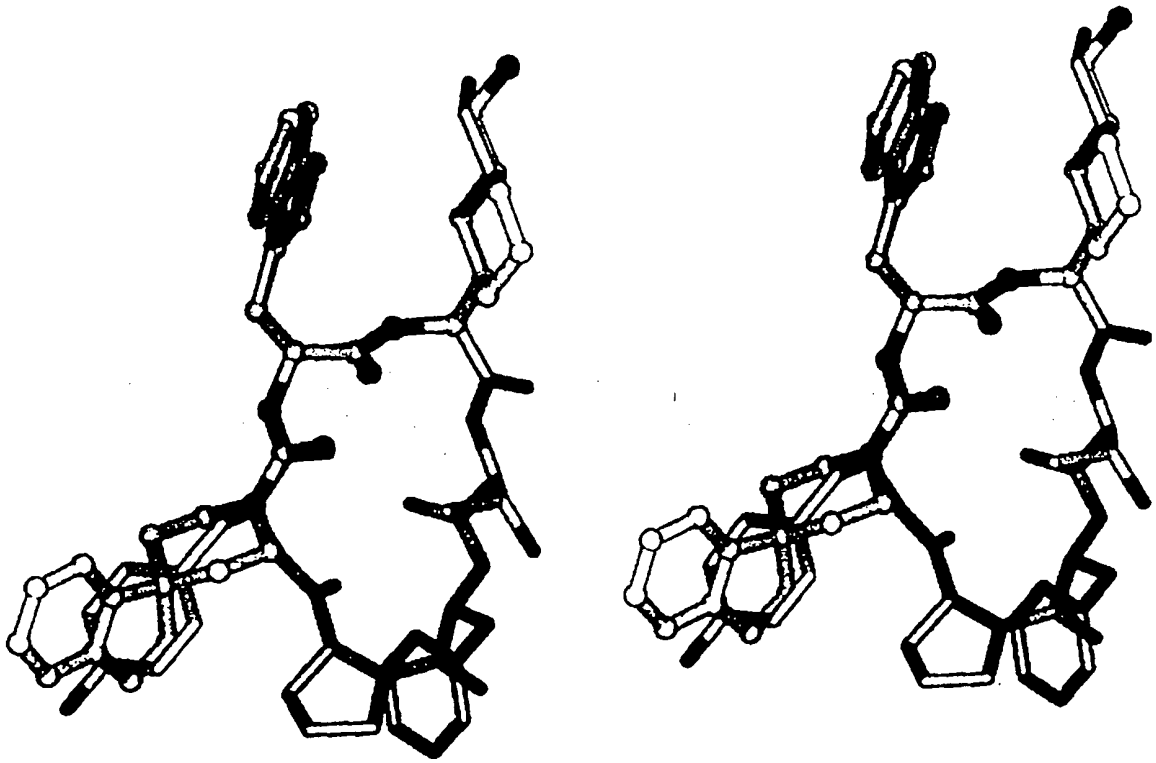


Fig. (36). Side-by-side stereo presentation of the structural overlay of **123** (ball-and-stick mode) onto the experimentally-derived conformation of **117** (stick-mode).

core was believed to mimic the additional lipophilic element referring to Phe^{6}/Phe^{11}, respectively. However, the racemic mixture of **119** (benzodiazepinone) showed an IC_{50} of 7 μM, and even after separation, the L- and D-Trp containing benzodiazepinone displaced the radioligand with IC_{50} of only 6.5 μM and 8.2 μM, respectively.

Similar affinities in the low micromolar range were obtained with peptidomimetics based on β-D-glucose scaffolding described by Hirschmann and Nicolaou at the end of the 80's and beginning of the 90's [250]. Molecular modeling studies carried out on the 3D structures of SRIF-14 **115** and analogues of L-363,377 **117** suggested that substituents at C-2, C-1, and C-6 of a β-D-glucose template resemble the orientational pattern of the β turn-forming amino acids of the somatostatin-derived peptides. The corresponding penta-substituted glucose **120** (Fig. (34) showed an IC_{50} of 15 μM.

In 1996, researchers from Rhône-Poulenc Rorer published a similar approach of *de-novo* designed peptidomimetics employing aza-sugar-based templates for the spatially controlled orientation of the pharamcophoric amino acid sidechains [251]. Independent of ring size and substitution

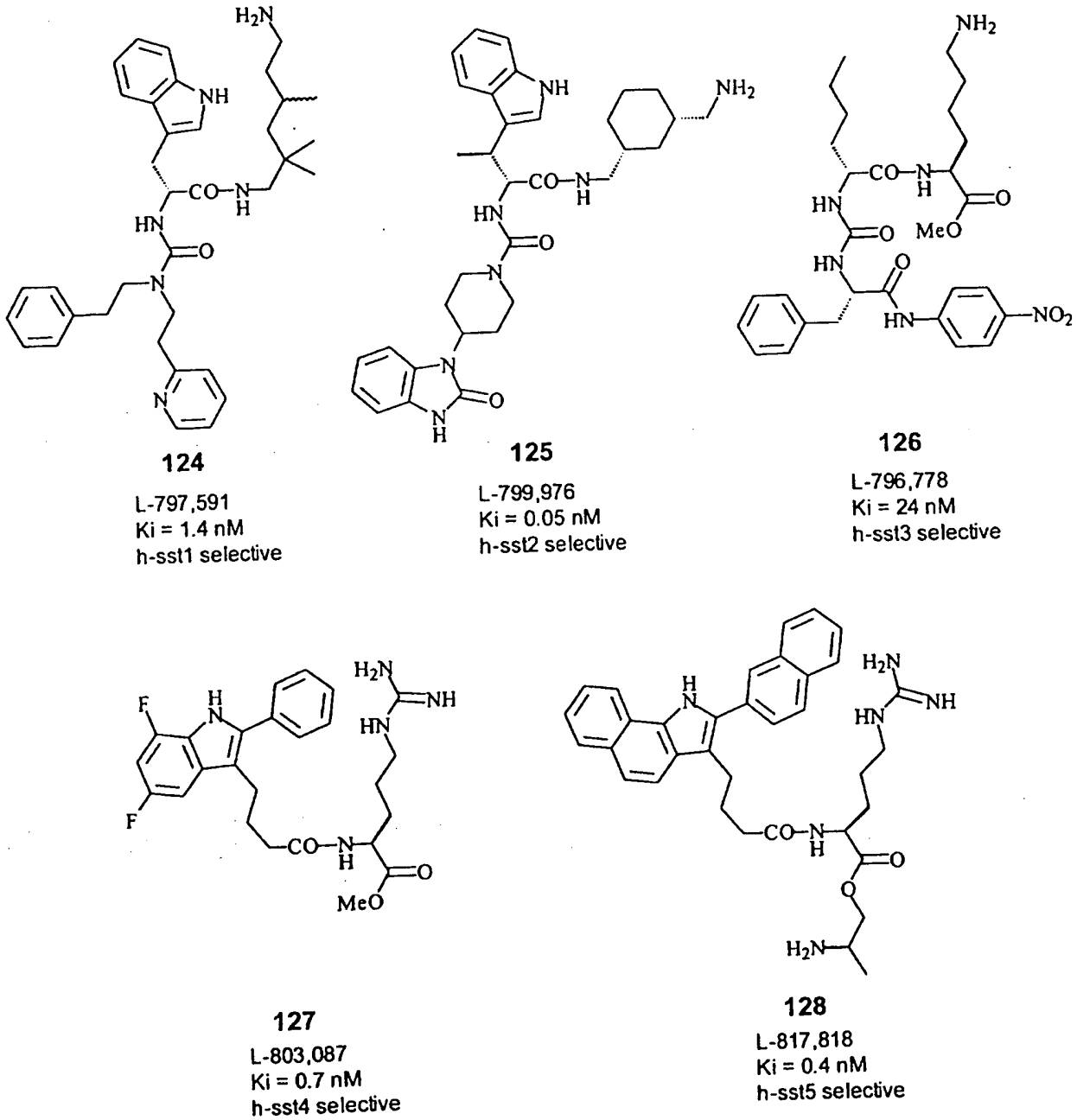


Fig. (37). For each somatostatin receptor subtype ($hsst_1$-$hsst_5$) highly selective compounds emerged from rationally designed combinatorial libraries.

pattern, all analogues showed weak affinity with IC_{50} values in the range of 10-15 μM (see for example **121**, Fig. (34)).

Over the last two years, scientists at the Merck Research Laboratories conducted a comprehensive program aimed to identify subtype-selective peptidomimetic compounds for each somatostatin receptor subtype (sst_1-sst_5) by following a rational design strategy using a combination of classical medicinal chemistry with modern combinatorial chemistry techniques [252-256]. The primary lead, L-264,930 **122** (Fig. (35)), that initiated that combined approach, was identified by a virtual screening of the Merck sample collection. The 3D structure of the cyclic hexapeptide L-363,377 **117** (Fig. (33)) served as spatial probe in that a geometric pattern, describing the arrangement of the pharmacophoric groups, was derived by means of molecular modeling. After similarity searches, in which the sidechains of residues Tyr^7-Trp^8-Lys^9 were given priority for the pharmacophore definition, L-264,930 **122** was uncovered with submicromolar affinity for the *h*sst_2 receptor.

This compound became the primary focus for medicinal chemistry and combinatorial chemistry at Merck. By constraining the floppy diamine chain with a 1,3-bis-aminomethyl-cyclohexane moiety the compound was optimized to yield L-054,264 **123** (Fig. (35), Fig. (36)) with an IC_{50} of 1.6 nM for the *h*sst_2 receptor and a more than 1000-fold selectivity over all other somatostatin receptor subtypes.

Simultaneously, L-264,930 **122** served as lead structure for a targeted combinatorial library. For library design the lead was dissected into three components, notably the central α-amino acid, the *C*-terminal blocking diamine, and the *N*-terminal blocking bulky urea-attached amine. The initial library was based on 20 α-amino acids, that were mainly analogues of Trp or carried modified aromatic sidechains. Additionally, 20 diamines were chosen in which the spacing between the two nitrogens varies between four and six atoms, also encompassing different ring topologies. The amine collection comprised 79 different entities that were biased towards piperidines and piperazines containing additional aromatic rings, so-called "privileged structures". A solid-phase mix-and-split protocol was used to synthesize more than 130000 compounds in complex mixtures that demanded a deconvolution strategy. After several rounds of iterative optimization employing classical analoging as well as follow-up libraries, five compounds **124** - **128** emerged with the desired activity and selectivity profile, in that each compound is highly selective for a distinct somatostatin receptor subtype (Fig. (37)).

This program impressively demonstrates the impact of an intelligent combination of structural rationales derived by comprehensive molecular modeling with the synthetic efficiency of current combinatorial chemistry techniques for lead finding attempts within modern medicinal chemistry.

A further example of a peptidomimetics-based library employing structure rationales for identification of subtype-selective somatostatin analogues was published recently by J. Ellman and co-workers (Fig. (38)) [257]. By decoration of a medium-sized heterocyclic β turn mimic with the Trp- and Lys-sidechain in positions *i+1-1+2* and vice versa, together with an additional amine building block in *i+3*, a remarkably small library of only 172 entities (22 amines, D/L-Trp-D/L-Lys, D/L-Lys-D/L-Trp) uncovered a *h*sst_5-selective compound **129** with an IC_{50} of 87 nM.

Bradykinin

Researchers at Sterling Winthrop considered angiotensin-converting-enzyme (ACE) inhibitors as templates for the design of BK B_2 receptor antagonists [258], since ACE degrades both, angiotensin II (AII) and BK by cleaving the Pro^7-Phe^8 amide bond. Therefore, an ACE inhibitor was considered to display properties or conformational similarities to BK, thus establishing a pharmacophoric link between ACE and BK receptors in that both macromolecules recognize similar steric and physicochemical features. In order to test this hypothesis, the ACE inhibitor Quinapril

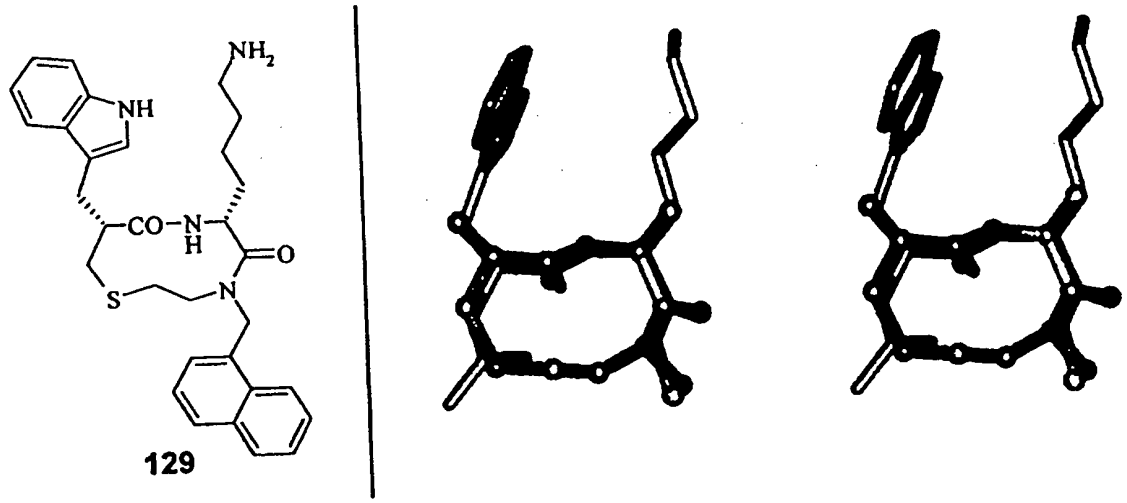
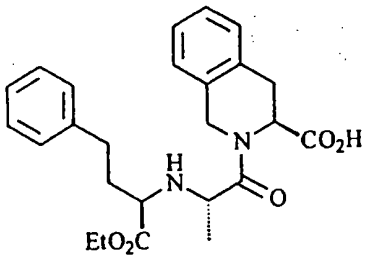


Fig. (38). Left: *h*sst_5-selective compound derived from a β turn-templated library; right: side-by-side stereo presentation of the structural superposition of the β turn mimic (ball-and-stick mode) onto the βII' turn portion of **117** (stick-mode).

130 (Fig. (39)) [259] was chosen as template for the design and synthesis of a series of *homo*Phe-Tic (Tic: tetrahydrisoquinoline) containing compounds. The diastereomeres of 131 (Fig. (39)) exhibit binding affinities in the micromolar range (K_i = 1 μM) in [^{3}H]BK binding studies with human IMR-90 fetal lung fibroblasts.



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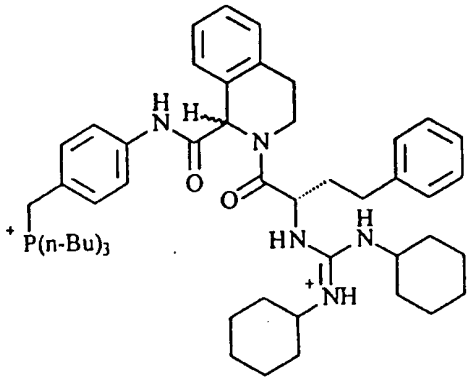


131

Fig. (39). Quinapril (130) served as template for the design of BK antagonists (e.g. 131).

Goodfellow *et al.* [260] followed a different approach in that they established a library based on a β turn template, CP-0597 132 (Fig. (40)) [261] which is a peptidic B_1/B_2 antagonist containing D-Tic and *N*-Chg (Chg: N-cyclohexylglyine) in *i+1* and *i+2* position of a βII' turn. Starting from that structural rationale, the peptidomimetic CP-2055 133 (Fig. (40)) was generated. Based on the 1,4-piperzine scaffold a combinatorial library has been designed to produce approximately 2500 *rationally directed diverse analogues* (RDDA), 134 (Fig. (40)).

This process led to the discovery of nonpeptide B_2 antagonists serving as lead compounds for traditional optimization. While the parent peptidic analogue CP-0597 132 shows an IC_{50} value of 0.33 nM, CP-2055 133 exhibits an IC_{50} value of about 55 μM on a cloned human B_2 receptor. CP-2458 is a further a member of the designed library 134 and inhibits human B_2 receptor binding (IC_{50}=4.1 μM) and BK-stimulated Ca^{2+} flux in human fibroblasts (IC_{50}=19 μM). Unfortunately, the chemical formula of the compound is not given explicitly in the publication.

Based on two structural templates (i) a cyclic hexapeptide BK antagonist 135 [262] and (ii) the nonpeptide BK antagonist WIN-64338 43 (Fig. (41)) [129], Dankwardt *et al.* [263] designed nonpeptide B_2 antagonists. While the hexapeptide served as structural template for the positioning of relevant functionality, WIN-64338 43 served as rigid scaffold for the design of a series of naphthylalanine containing derivatives, none of which showed improved affinity for the B_2 receptor when compared to WIN-64338 43 (K_d = 44 nM. Substitution of the phosphonium group against the corresponding ammonium moiety resulted in a two-fold decrease in affinity for the B_2 receptor. However, the proposed structural superposition of the cyclic hexapeptide 135 with the blocked amino acid derivative 43 provided a pharmacophore hypothesis that enabled Dankwardt and coworkers to design moderately active compounds and might serve as structural blueprint for further design attempts[263].

Neurokinin

The structural feature of a reverse β turn has emerged to a general design principle underlying a variety of GPCR antagonist projects. β truns play an important role in recognition phenomena as documented e.g. for somatostatin and NKA which bind to their receptors in a proposed β turn conformation. Therefore Horwell *et al.* [264] at Parke-Davis

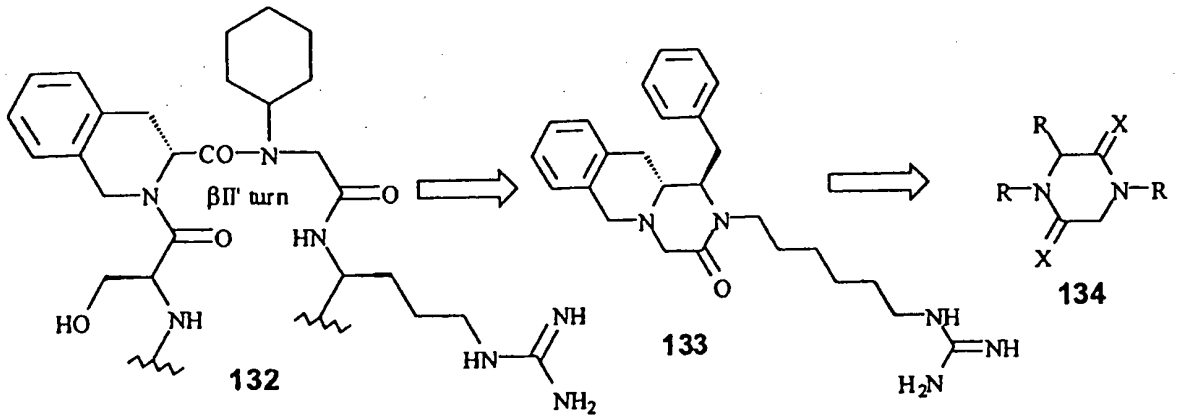


Fig. (40). Design strategy of BK antagonists following the "*rationally directed diverse analogues*" approach.

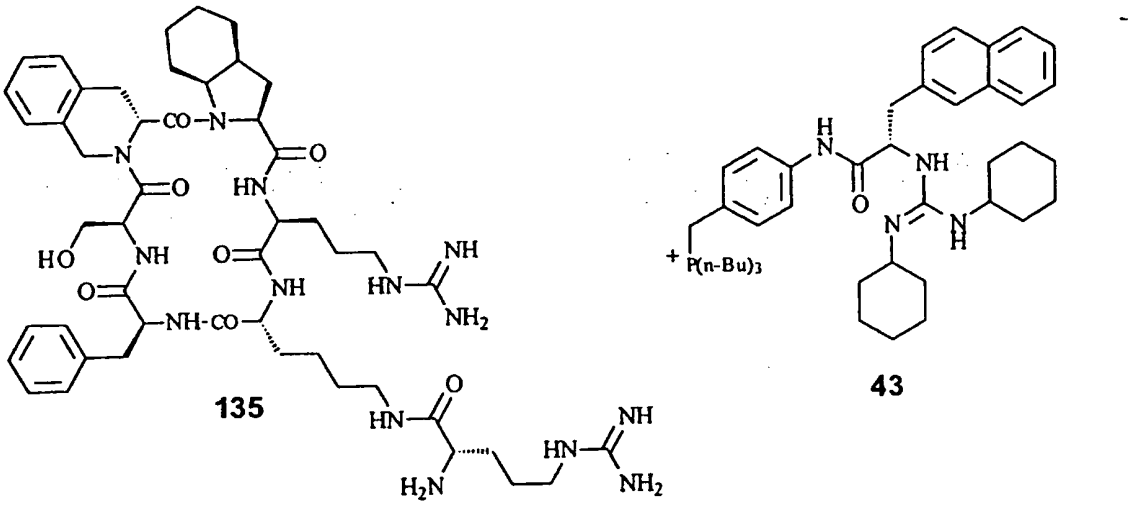


Fig. (41). Rationally designed BK antagonists.

decided to employ β turn mimetics for the design of compounds with affinity for the NK_2 receptor. Starting from the x-ray structure of MEN-10627 **136** (Fig. (**42**)) [265], a cyclic hexapeptide displaying high NK_2 affinity, a pyrrolidine-based Trp-Phe dipeptide mimetic **137** has been designed (Fig. (**42**)).

The Trp-Phe dipeptide scaffold mimics the Trp-Phe fragment in the central portion (*i+1, i+2*) of a βI turn within the cyclic hexapeptide which folds into a βI/βII turn conformation. Although the indole and benzyl sidechains of both compounds superimpose satisfactory, **137** did not show significant NK_2 receptor affinity. The lack of affinity has been attributed to the misfit of the dipole moments of both molecules. In order to address this problem in more detail, a further Trp-Phe dipeptide mimetic **138** (Fig. (**42**)) has been designed by computer-assisted molecular modeling identifying a 2-azabicyclonorbornan spacer to be more favourable compared to the pyrrolidine (Fig. (**43**)).

Comparison of the binding affinities revealed that the conversion of the hexapeptide to a dipeptide unit results in the loss of high binding affinity (MEN10627 **136**: IC_{50}=0.079 nM (NK_2); **137**: IC_{50}=14% @ 10 μM (NK_2); **138**: IC_{50}=31% @ 10 μM (NK_2)) studied by displacement assays with [^{125}I]NKA in hamster urinary bladder. On the other hand, [^{125}I]BH-SP displacement from NK_1 in human IM-9 cells of MEN-10627 **136** (IC_{50}=0.8 μM) is retained by **137** and **138** with IC_{50} values of 3.7 μM and 6.7 μM, respectively. Interestingly, the dipeptide mimetics exhibit some binding affinity to human NK_3 receptors stably expressed in CHO cells shown by replacement of [^{125}I]-[MePhe^7]NKB (**137**: $IC_{50}(NK_3)$=3.5 μM; **138**: $IC_{50}(NK_3)$=35% @ 10 μM) while the parent hexapeptide exhibited no NK_3 affinity at all.

Only recently, Porcelli *et al.* [266] presented the design of a SP antagonist based on a cyclic pentapeptide with the chirality sequence following a $D^1L^2D^3D^4L^5$ pattern. The

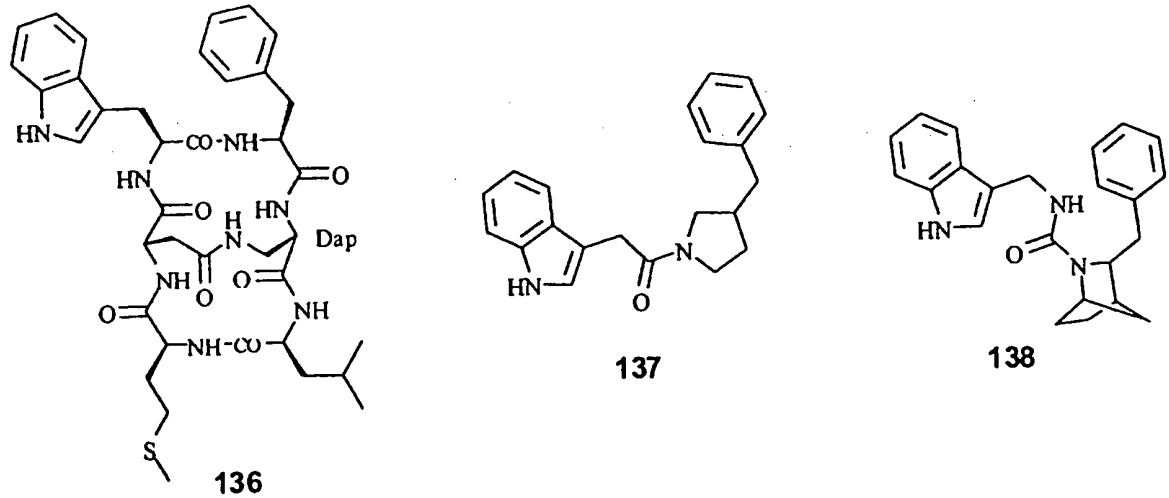


Fig. (42). Peptide structure-derived rationales were used to design non-peptide NK antagonists (Dap: 2,3-diaminopropanoic acid).

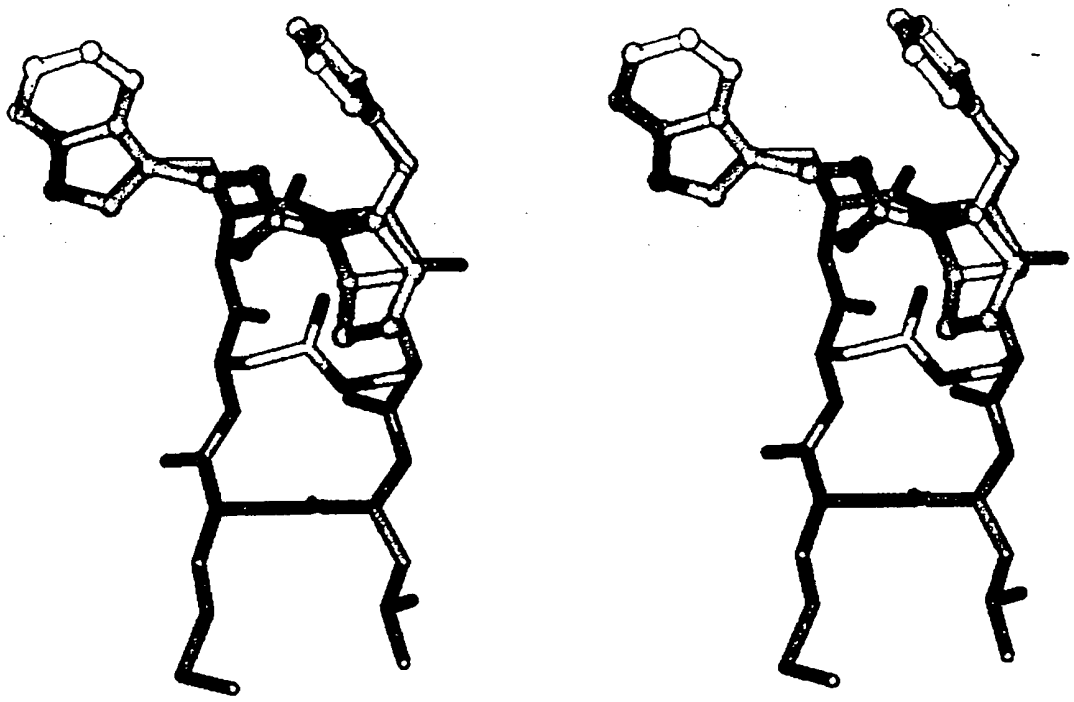
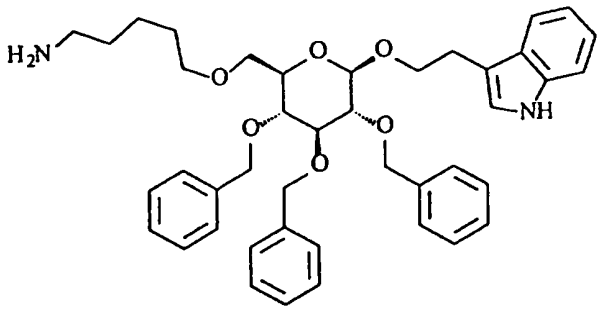


Fig. (43). Side-by-side stereo presentation of the structural overlay of 138 (ball-and-stick-mode) onto the x-ray structure of 136 (stick-mode) within the turn corresponding portion.

authors suggest this scaffold as a generic template to design antagonists also for other members of the GPCR family. This suggestion is the logical consequence of the fact that among potent GPCR antagonists the same unique skeleton is found among other representatives of antagonists for peptide-binding GPCRs, e.g. the natural pentapeptide BE-18257B (*cyclo*-(D-allo-Ile-Leu-D-Trp-D-Glu-Ala-)) and its synthetic analogue BQ-123 (*cyclo*-(D-Val-Leu-D-Trp-D-Asp-Pro-)) [267], a prominent ET_A antagonist. Both cyclic pentapeptides follow the chiral sequence pattern of DLDDL. The solution structure of BQ-123 [268] exhibits a typical $\beta II/\gamma_i$ turn arrangement characteristic for this class of molecules. Based on the same structural template, Porcelli *et al.* designed a SP antagonist, ITF-1565 (*cyclo*-(D-Trp^1-Pro^2-D-Lys^3-D-Trp^4-Phe^5-)) which inhibits NK_1-mediated SP-induced contraction of the rabbit caval vein. ITF-1565 only shows modest NK_2 activity and was inactive in ET_A assays. ITF-1565 exhibits a $\beta II/\gamma$ turn arrangement with Pro^2 in *i+1* and D-Lys^3 in *i+2* position of the β turn and Phe^5 in the central position of the γ turn. Interestingly, the authors succeeded to superimpose the sidechain functionalities of D-Trp^4, Phe^5 and D-Trp^1 within ITF-1565 well onto the indole and benzyl rings within a β-D-glucose derived SP antagonist 139 (Fig. (44)).



139

Fig. (44). Glucose-based peptidomimetic NK analogue.

Luteinizing Hormone-Releasing Hormone

The decapeptide amide Luteinizing Hormone-Releasing Hormone (LHRH, Table 1) [269], *p*Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, is released from the hypothalamus and stimulates the anterior pituitary gland resulting in the secretion of the gonadotropins luteinzing hormone (LH) and follicle-stimulating hormone (FSH). LHRH, also termed gonadotropin-releasing hormone, plays an important role in the regulation of reproductive functions, thus rendering its synthetic analogues useful tools for the treatment of endocrine-based diseases like prostate and breast cancer, endometriosis, uterine leiomyoma, and precocious puperty [270]. Even though LHRH agonists proved to be useful in the treatment of the above mentioned diseases [271-273], research has also focused on the development of potent and safe antagonists.

Recently, Takeda presented a substituted 4-oxothieno[2,3-*b*]pyridine as a highly potent and orally active nonpeptide antagonist of the human LHRH receptor [274]. Again, this research program was based on the structural characteristics of a β turn suggested as the dominant conformational feature within [5-8]LHRH (Fig. (45)) [272,273].

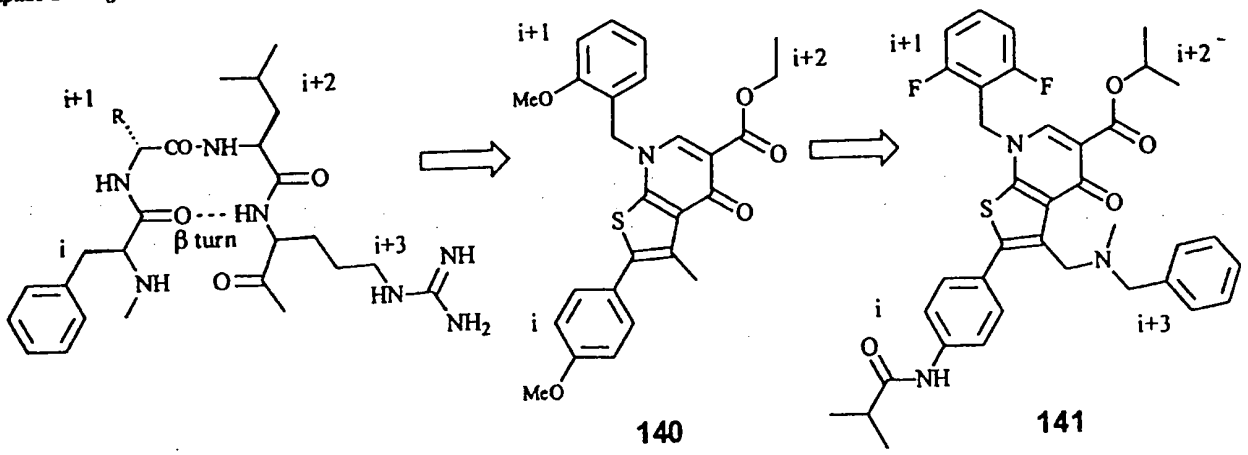


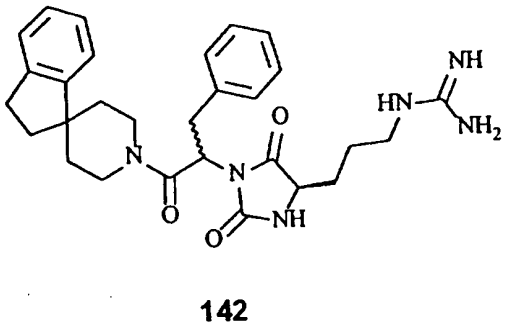
Fig. (45). β turn-derived design strategy uncovered highly active non-peptide LHRH analogues.

The β turn is considered to represent the bioactive conformation of LHRH in the receptor-bound state. Therefore, the structural element of a β turn was attempted to be transferred onto a rigid scaffold which mimics the β turn and can be decorated with the crucial functionalities, thus positioning them into the receptor-complementary orientation (Fig. (45)). For this purpose, a directed screening approach was initiated aimed to uncover compounds showing similarity to the turn template. The screening towards the inhibitory effect on the specific binding of [^{125}I]leuprolelin to human LHRH receptor [275] expressed in CHO cells resulted in the initial lead compound **140** (Fig. (45)) [274].

This compound was structurally compared to the hypothesized β turn arrangement and changed in order to fulfil the structural requirement imposed by that template, e.g. substituting Gly by hydrophobic D-amino acids increased activity presumably due to stabilization of the β turn by introducing a D-amino acid into the *i+1* position of the β turn. Subsequent modifications finally led to the discovery of T-98475 **141** (Fig. (45)) exhibiting an IC_{50} value of 0.2 nM for the binding to the cloned human LHRH receptor. Further, T-98475 **141** shows inhibitory effects on LHRH-stimulated LH release in functional *in vitro* and *in vivo* assays. Thus, T-98475 **141** is a good candidate of a new class of therapeutics for the treatment of LH-induced dysfunctions in sex-hormone-dependent pathologies.

C5a

The 74 amino acid peptide C5a (Table 1) is released after activation of the complement system at sites of inflammation



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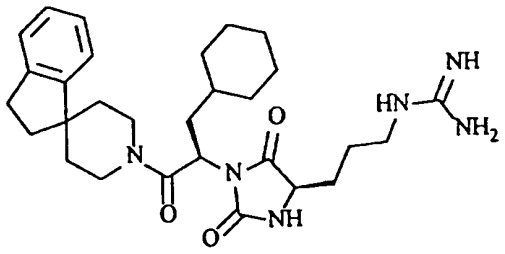

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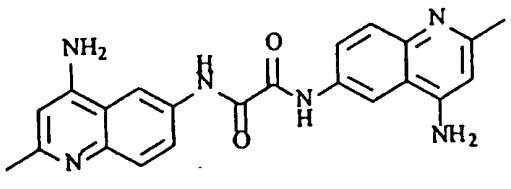

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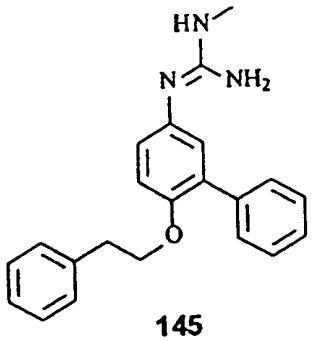

145

Fig. (46). C5a analogues.

by proteolytic cleavage of the complement factor C5 [276]. The hormone-like peptide anaphylatoxin, C5a, acts as chemotaxin by attracting and promoting the degranulation of granulocytes and macrophages during immune response [277,278]. Inappropriate activation of C5a results in a number of inflammatory diseases including rheumatoid arthritis [279], Alzheimer's disease [280], ischemic heart failure [281], psiorasis [282], atheriosclerosis [283], and adult respiratory distress syndrome (ARDS) [284]. In this sense, agents preventing the interaction of C5a and its receptor, C5aR, would be useful for inhibition of the pro-inflammatory function of C5a, thus, being a useful therapeutic in the treatment of chronic inflammatory disorders induced by activation of the complement system and the release of C5a [285,286]. The binding of the small protein C5a to its receptor is characterized by two interaction sites. A two-site model has been proposed localizing the major binding epitope for the ligand C5a in the extracellular *N*-terminal region of the receptor, while the second binding cavity is located in the core of the transmembrane helix bundle, obviously serving as the "activating binding site" recognizing the *C*-terminal octapeptide of the ligand [287,288]. Starting from the sequence of the native ligand a number of peptide-based antagonists were discovered which have been reviewed only recently by Wong *et al.* [289]. Obviously, the development of a nonpeptide antagonist in this filed is a major challenge since research revealed only low molecular weight compounds acting as C5a agonists or at least partial agonists over the last two decades.

Merck identified an initial lead **142** (Fig. (46)) by screening an in-house sample collection for the displacement of [^{125}I]C5a from human neutrophil membrane preparations which served for further optimization [290].

The spiroindane-bearing hydantoin **142** has been modified by introduction of a cyclohexylmethyl group instead of the benzyl residue resulting in compound **143** (Fig. (46)) which exhibits an IC_{50} value of 0.3 μM.

Surprisingly, functional receptor assays revealed that all compounds of this series with affinity for C5aR showed an agonistic potential. The only nonpeptide antagonists have been reported by Merck investigating 4,6-diaminoquinolines (**144**) [291] and Rhône-Poulenc Rorer identifying a phenylguanidin by random screening (**145**, IC_{50}=0.8 μM) (Fig. (46)) [292].

As random screening techniques have not brought the expected success, rational design would offer an alternative in the lead finding process for C5a antagonists. Based on the results of conformational studies of cyclic pentapeptide ET antagonists, BE-18257B and BQ-123 [293,294], Wong and co-workers [295,296] followed the same strategy as presented by Porcelli *et al.* [266] for the design of the SP antagonist, ITF-1565. BQ-123, *cyclo*-(D-Val^1-Leu^2-D-Trp^3-D-Asp^4-Pro^5-) and ITF-1565, *cyclo*-(D-Trp^1-Pro^2-D-Lys^3-D-Trp^4-Phe^5-) follow an identical chirality pattern of $D^1L^2D^3D^4L^5$ leading to a $\beta II/\gamma_{(i)}$ turn arrangement with L^2-D^3 in *i+1* and *i+2* position of the β turn and L^5 in the central position of an (inverse) γ turn. The strategy seems also to be applicable to C5a, since the *C*-terminal-derived C5a antagonist NMe-Phe-Lys-Pro-D-Cha-Trp-D-Arg (Cha: cyclohexylalanine) shows a well defined structure in solution in which the lysine sidechain is in close proximity to the D-arginine carboxylate. Ring closure resulted in a backbone-to-sidechain cyclized peptide, *cyclo*-Ac-Phe-(Orn-Pro-D-Cha-Trp-D-Arg-) (brackets indicate the sidechain-to-backbone mode of cyclization, Orn-NH$^{\varepsilon}$-CO-D-Arg) with an IC_{50} value of 9.28 μM for the displacement of [^{125}I]C5a from human polymorphonuclear (PMN) cells. Conformational analysis revealed a γ turn with Pro in the central position stabilized by a hydrogen bond between the flanking amino acids, Orn-CO···HN-D-Cha, together with a "pseudo" βII turn involving D-Cha-Trp-D-Arg-Orn defined by a second hydrogen bond between D-Cha-CO···H$^{\varepsilon}$N-Orn. This is consistent with ϕ_{i+1}/ψ_{i+1} and ϕ_{i+2}/ψ_{i+2} dihedrals of Trp and D-Arg (-58°/90°; 69°/-3°) confirming a β turn type II (ideal values: -60°/120°; 80°/0°) arrangement [295]. More detailed SAR studies showed that the L-Arg containing isomer is much more active than the D-Arg congener (IC_{50}=20 nM; inhibition of C5a-induced release of myeloperoxidase from PMNs). The NMR-derived solution structure reveals an inverse γ turn (γ_i) involving D-Cha-Trp-Arg stabilized by a hydrogen bond between D-Cha-CO···HN-Arg [296].

CONCLUSION

This review was intended to highlight not only the relevance of the GPCR superfamily for drug development purposes during the last decade, but also the tremendous potential of that particular target class for future medicinal chemistry programs aimed to uncover new ligands for peptide-binding GPCRs. Especially the cross-fertilizing combination of ligand-derived structure rationales with the dramatically enhanced efficiency of automated synthesis and combinatorial chemistry will enable pharmaceutical research to identify new chemical entities more rapidly. Even though we have witnessed a technology-based quantum leap forward in efficiency within medicinal chemistry in the late 1990's, the vigorous search for novel GPCR genes within e.g. the human genome has far outpaced the identification of novel endogenous and exogenous ligands. The identification of these ligands remains one of the most challenging tasks in modern pharmacology. The number of GPCRs for which endogenous or exogenous ligands are unknown today continues to increase, thus offering modern pharmaceutical research new opportunities in that entirely new drug targets associated with innovative therapeutic principles emerge. In this context, new low-molecular weight ligands for these orphan receptors will undoubtedly lead to novel insights into the complexity of numerous poorly understood human disorders. Consequently, targeted medicinal chemistry approaches towards members of the GPCR family will facilitate the understanding of the precise physiological role of orphan receptors as well as produce new compounds as qualified lead structures for clinical development.

Concluding, the field of GPCR research is clearly expected to grow dramatically due to the progress that will be made in the human genome initiative, demanding increased contributions from medicinal chemistry in order to provide new pharmacological tools as well as new leads for the development of new drugs.

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